Kinetics and Mechanisms of Oxidation of Hemoprotein Model Compounds[1]*

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Received December 7, 1976

The kinetics of reaction of oxyheme complexes with dithionite ion and with deoxyheme were studied. Because rates of autooxidation of oxyheme complexes were inversely proportional to oxygen pressure and proportional to the square of the total heme concentration, it was concluded that the reaction proceeds through Heme--OO-Heme as suggested by Cohen and Caughey. The direct reaction of dithionite ion with oxyheme complexes accords with the $Fe^+O_2^-$· formulation of the iron-oxygen bond.
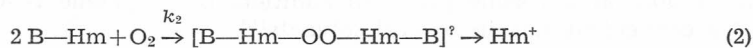
INTRODUCTION

The autoxidation of hemes to hemins by molecular oxygen,

$$Fe^{II} \text{ (porphyrin)} + O_2 \rightarrow [Fe^{III} \text{ (porphyrin)}]^+ + \text{oxygen compounds} \qquad (1)$$
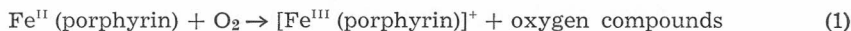
is an important chemical and biological process. In oxygen transport proteins, such as myoglobin, this process destroys the oxygen binding ability of the iron and must be avoided or greatly retarded[3-5]. In oxidases something like this process produces ROOH or ROH instead of H_2O_2 or water and is therefore required[5]. When simple heme compounds are exposed to oxygen in water or other solvents they are »instantaneously« oxidized[6] (eq. 1)[5] in contrast to the heme in myoglobin which is oxidized over a period of many hours[3,7-10]. An understanding of the mechanisms by which this oxidation takes place would help to clarify both the resistance of myoglobin to oxidation and the possible use of the oxidation intermediate in biological oxidations.

Two types of mechanisms have been sugested for reaction 1,[4,8,9] a bimo-lecular reaction (2) and an acid-catalyzed unimolecular mechanism (3). [We will represent an iron porphyrin as Hm and use either Hm or Hm^{II} to represent iron(II) and Hm^{III} or Hm^+ to represent iron(III). In kinetic equations HM $\equiv \Sigma$ heme in solution.]

$$2\,B\text{—Hm} + O_2 \xrightarrow{k_2} [B\text{—Hm—OO—Hm—B}]^? \rightarrow Hm^+ \qquad (2)$$

$$B\text{—Hm} + O_2 + H^+ \xrightarrow{k_3} [B\text{—Hm—OOH}]^? \rightarrow Hm^+ \qquad (3)$$

Although reaction 3 has not been studied in detail[7], it is strongly suggested by the increased rate of autoxidation of hemes and hemoproteins in acid[9c]. Reaction 2 has been suggested to accommodate rates (of autoxidation of heme--pyridine complexes) which are second-order in heme[7,8].

Following the discovery that excess pyridine retards autoxidation of hemes in solution, Cohen and Caughey[9a] studied the kinetics of protoheme autoxidation in benzene solutions of pyridine. Under these conditions no oxygen complex can be observed. For the overall reaction
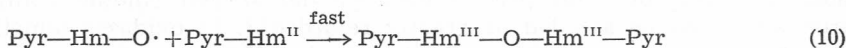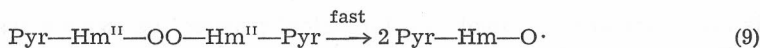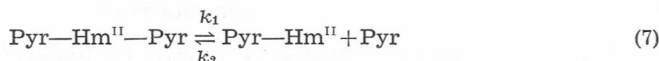
$$4\,Hm(Pyr)_2 + O_2 \rightarrow 2\,Pyr—Hm—O—Hm—Pyr \tag{4}$$

three different kinetic orders were determined. At high pyridine and low oxygen concentrations

$$\frac{d\,Hm^+}{dt} = k\,{}^5_{obsd}\,\frac{[O_2]\,[Hm]^2}{[Pyr]^2} \tag{5}$$

whereas, at high oxygen and low pyridine concentrations

$$\frac{d\,Hm^+}{dt} = k\,{}^6_{obsd}\,[Hm] \tag{6}$$

These results were interpreted in terms of the known equilibrium (7) and the formation of an unstable peroxide

$$Pyr—Hm^{II}—Pyr \underset{k_2}{\overset{k_1}{\rightleftharpoons}} Pyr—Hm^{II} + Pyr \tag{7}$$

$$2\,Pyr—Hm^{II} + O_2 \rightleftharpoons Pyr—Hm^{II}—OO—Hm^{II}—Pyr \tag{8}$$

$$Pyr—Hm^{II}—OO—Hm^{II}—Pyr \xrightarrow{fast} 2\,Pyr—Hm—O\cdot \tag{9}$$

$$Pyr—Hm—O\cdot + Pyr—Hm^{II} \xrightarrow{fast} Pyr—Hm^{III}—O—Hm^{III}—Pyr \tag{10}$$

The reactions were carried out under conditions of no oxygenation whereas autoxidation of heme proteins usually occurs under oxygenation conditions[10]. It is therefore interesting to study the autoxidation of simple oxyheme compounds.

We have recently prepared a series of hemoprotein active site model compounds *1—4* in which the »proximal« base is covalently attached to the heme[11a]. The compound *1* has spectra almost identical to those of reconstituted deuteroheme myoglobin as the deoxyheme, hemin, oxygen complex and carbon monoxide complex[11b,c]. Furthermore, when suspended in aqueous buffer at pH = 7.3 with two percent cetyltrimethylammonium bromide the compounds *1* and *2* bind oxygen with the same binding constant (1.7×10^6 l/M) as does myoglobin at the same pH[11a]. In addition, the oxyheme *1—O$_2$* is autoxidized at a convenient rate in several solvents[11d].

We are thus able to explore possible direct interaction of the oxyheme with substrates in an effort to discover how complexes of this kind either activate or deactivate oxygen for oxidation of such substrates. An idealized picture of possible oxidation by hemoproteins such as cytochrome *P450* is shown below where S = substrate.

$$B-Fe-O-O \xrightarrow{H_3^+O} \xrightarrow{H_2O} B-Fe^+ + SO + H_2O$$
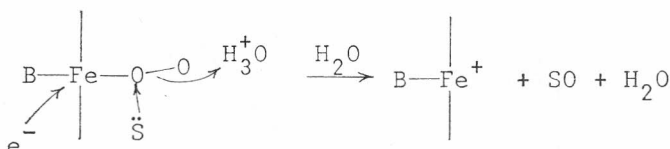
To produce an oxidant of sufficient reactivity to oxidize a hydrocarbon C—H bond some extra activation by electron donation and electrophilic (*e. g.*, H_3^+O) attack seems to be required. However, the three reactants proposed to react with the oxyheme are possibly connected, and it is conceivable that, as the reduction potential (reductive reactivity) of the substrate S is increased, less of the other activation by H_3^+O or e^- would be required.

We therefore began by studying the reactions of the oxyhemes 1—O_2 etc. with reductants in neutral solution. According to the mechanism suggested in equation 2, the deoxyheme 1 can serve as the substrate, S, perhaps without the involvement of the e^- or H_3^+O in equation 11. We have therefore reinvestigated this mechanism under oxygenation conditions and have also discovered a direct reduction of the oxyheme 2—O_2 by dithionite ion.

EXPERIMENTAL

The heme compounds *1, 2* and *3* have been described[11a,b,c]. All were prepared by coupling the appropriately substituted base with a pyrroporphyrin or mesoporphyrin mono or diacid, using thionyl chloride or pivaloyl chloride activation. Detailed procedures, to be published shortly, are available from the authors.

Dimethyl formamide was purified by the method described by Fieser and other solvents were reagent grade.

Stopped flow kinetics measurements were made on a Durrum stopped-flow spectrophotometer exactly as described by Gibson[12]. Slower oxidation rates were carried out using solutions of the heme compounds which had been reduced by the Pd/CaH_2 method[19] (in DMF or DMF/H_2O), the Pd black method[9] (in benzene), or with a stoichiometric quantity of sodium dithionite[11c] (aqueous suspension or aqueous alcohol). Solutions were transferred to a closed cuvette under argon ofr static or slow kinetic measurements and to syringes for stopped-flow kinetic measurements.

RESULTS

When the dipyridine heme compound *3* is dissolved in benzene, it shows no sign of oxygenation at 1 atm oxygen but is oxidized cleanly to the Hm^{III} compound with rates indicated by the plot shown in Figure 1. The reaction is accurately second-order in heme and is accelerated by increasing oxygen pressure. This result resembles that of Cohen and Caughey at high pyridine concentration[9a].

Under 1 atm of oxygen at —45 ^{0}C in methylene chloride the monoimidazole compound *1* forms an oxygen complex which is stable against oxidation for several hours. However, at lower oxygen pressures, the oxidation is faster, having approximate half times for oxidation of 30 min at 100 Torr O_2, 25 min at 20 Torr and about 16 min at 2 Torr O_2 with heme concentration of 4.4×10^{-5} M. This result suggests that *oxygen retards oxidation* of this synthetic myoglobin active site just as it does in myoglobin itself[10].

Because these results were difficult to reproduce accurately in methylene chloride, the reaction was studied further in dimethyl formamide (DMF) at 15 ^{0}C[11d] where the oxidation is slower. Solutions of the compound *1* at 1.6×10^{-5} M in dry DMF were equilibrated quickly with oxygen at known pressures and
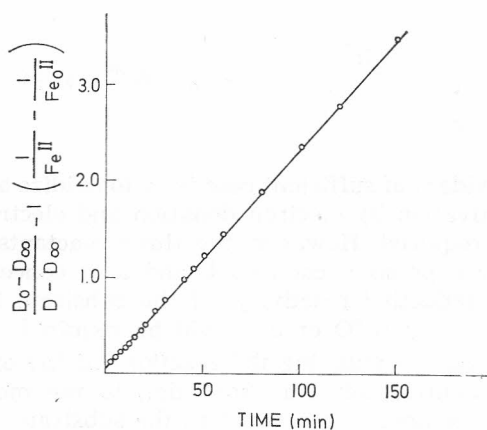
Figure 1. Autoxidation of di-pyridine mesoherme *3* in benzene at 25 °C. Conc. of *3* -5.5 × 10⁻⁵ M. Absorbance (D) followed at 547 nm. Oxygen pressure 1 atm.

the change in the oxyheme Soret peak at 408 nm[11a] followed. Plots of $1/Hm - 1/Hm^0 = (D_0 - D_\infty / D - D_\infty - 1)$ *versus* time were accurately linear to 60——80% reaction, as shown in Figure 2. The second-order rate constants obtained from these plots are listed in Table I. The near constancy of the product $P_{O_2} \times k_{obs}$ indicates that the reaction rate is inversely proportional to oxygen pressure above about 350 Torr. Because the autoxidation must have zero rate at zero oxygen pressure, the rate should go through a maximum at approximately the $P_{1/2}(O_2)$ of the heme or about 0.5 Torr. We have been unable to
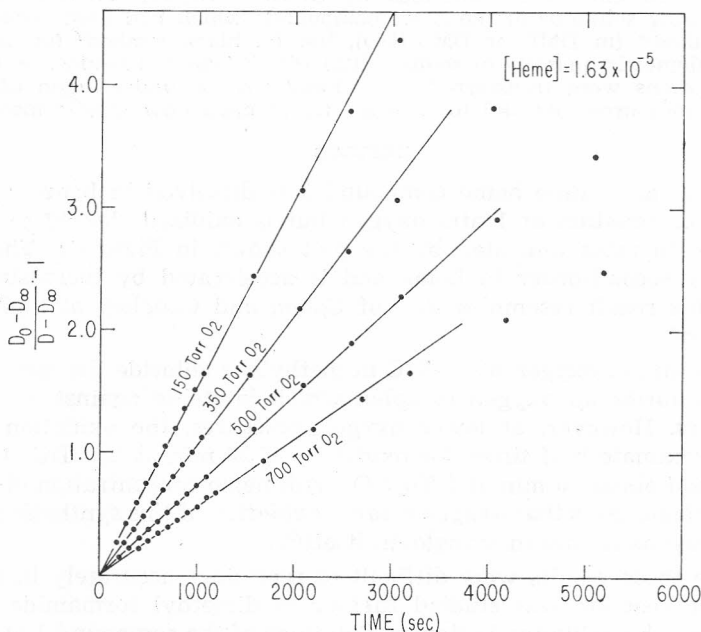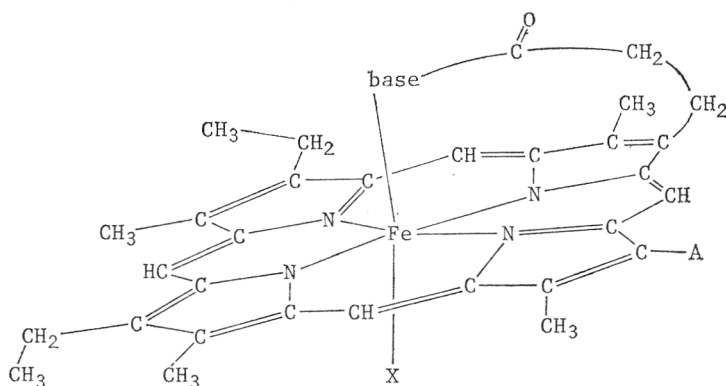

Figure 2. Autoxidation of pyrroheme-N-[3-(1-imidazolyl)propyl]amide, *1*, in dry DMF at 15 °C Conc. of heme 1.63 × 10⁻⁵ M.

TABLE I

Rate constants for autoxidation of 1 by oxygen at 15 °C in anhydrous dimethyl formamide

P_{O_2} (Torr)	k ($M^{-1} s^{-1}$)	$P_{O_2} \times k_{obsd}$ ($\times 10^{-3}$)
700	26	18
500	44	22
350	60	21
150	84	13
(100)*	(120)*	(12)*

*. In methylene chloride at -45 °C $4.4 \cdot 10^{-5}$ M heme. At 15 °C the oxidation was too fast to measure.

Compound	Base	A	X
1	$-NH(CH_2)_3-N\underset{}{\bigcirc}N$	H	
2	$-NH(CH_2)_3-N\underset{}{\bigcirc}N$	$-(CH_2)_2\overset{O}{C}-OMe$	
3	$-O(CH_2)_3-\bigcirc N$	$-(CH_2)_2\overset{O}{CO}(CH_2)_3-\bigcirc N$	Pyr of A

get reproducible oxidation rates at low oxygen pressures, but have repeatedly observed rather rapid autoxidation of compounds like *1* at low oxygen pressure.

Solvent and Acidity Effects

When a solution of 10^{-5} M heme *1* in a solution composed of 60% methylene chloride, 35% methanol and 5% water by volume was reduced with a slight excess of sodium dithionite, cooled to —45 °C and 1 atm oxygen admitted, the oxyheme spectrum shown in Figure 3 (1) was obtained. However, in contrast to the several hour stability in pure methylene chloride this oxyheme was oxidized in about 10 min (see Figure 3). We have also reported previously that in water-CTAB suspension, methylene chloride, or alcohols, autoxidation of *1*—O_2 occurred at 20 °C before a spectrum could be obtained[11d] (in a few seconds).

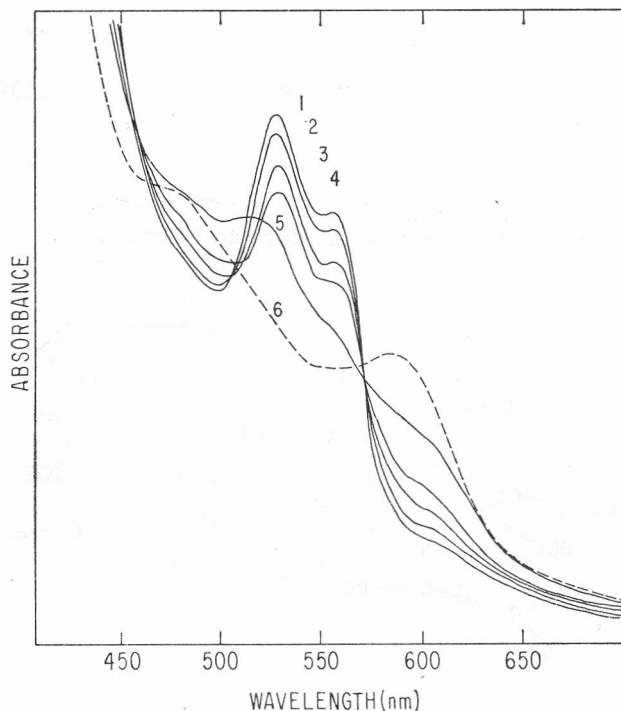Figure 3. Autoxidation of *1* in 60% CH_2Cl_2, 35% MeOH, 5% H_2O at —45 °C. 1) 30 s after admitting 1 atm oxygen to reduced *1*, $\lambda_{max} = 528$, 555 nm (compare with reference 11d). 2) 2 min. 3) 4 min. 4) 10 min. 5) Fully oxidized at —45 °C. 6) After warming 5 to 20 °C.

Contrary to these results, autoxidation of the same oxyheme compound at the same concentration in DMF was slow (Table I), showing no appreciable oxidation in 5 min at 0 °C. When 20% water was added to the solution of the oxyheme *1*—O_2 in DMF, autoxidation was not significantly accelerated. The effect was only to dilute the solution and lower all the absorbances (Figure 4).

These results suggest that water is not sufficient to cause rapid autoxidation if the solvent is somewhat basic as in DMF.
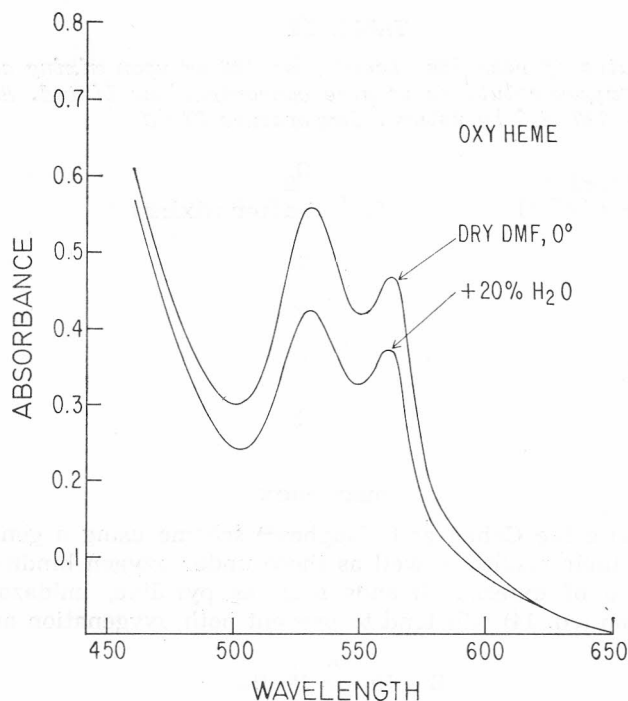
Figure 4. Spectra of the oxyheme formed by the addition of 1 atm oxygen to a DMF solution $(2 \times 10^{-5}$ M) of 1 at 0 °C (upper curve) and the spectrum after adding 20% water (lower curve). The aqueous solution displayed an autoxidation half time of about 30 min, similar to that of a DMF solution at this temperature and concentrations.

Reduction of Deoxyheme 1 and Oxyheme 1-O_2 by Dithionite Ion

In order to employ the Gibson[12] oxygen pulse technique to determine oxygen dissociation rates from, e. g., 1—O_2, we explored the kinetics of reaction of dithionite ion with the hemes 1 and 2 in the presence and in the absence of oxygen. In the absence of oxygen but in the presence of 2.5×10^{-5} to 10^{-4} M carbon monoxide the reduction was first order in heme and in dithionite ion. The second-order rate constant for the reaction (12) was estimated to be $k_{12} = 3 \times 10^4$ M^{-1} s^{-1} in aqueous dimethyl formamide.

$$1^{III} + S_2O_4 = \overset{k_{12}}{\underset{CO}{\rightarrow}} 1—CO \qquad (12)$$

The reaction of a heme 2-dithionite mixture with oxygen in the same $2:1$ DMF-water solvent proceeds at a rate which is sufficiently slow to allow the oxyheme 2—O_2 to be formed. In addition, the spectrum of the first formed intermediate accords with that of 1—O_2 and reverts to a spectrum of 1 at rates shown in Table II.

Unlike the oxygen pulse kinetics of hemoproteins[12] this reaction is dependent upon concentration of dithionite ion.
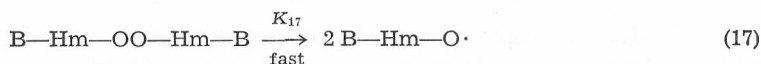
TABLE II

Stopped-flow rates of reaction observed at 422 nm upon mixing equal volumes of heme 2 and oxygen solutions to give concentrations listed. Heme conc. 7.5 μM, solvent 2:1 DMF:H_2O by volume. Temperature 23 °C

sodium dithionite (10^4 μM after mixing)	O_2 (10^4 M after mixing)	k_{obsd} (s^{-1})
45	4	286
30	2	217
8	2	154
4	2	126

DISCUSSION

If we rewrite the Cohen and Caughey[9a] scheme using a general base, B, we can derive their results as well as those under oxygen binding conditions. In the presence of external ligands such as pyridine, imidazole, CO, *etc.*, hexacoordination (eq. 14) will tend to prevent both oxygenation and oxidation.

$$B + Hm \overset{K_{13}}{\rightleftharpoons} B—Hm \tag{13}$$

$$B' + B—Hm \overset{K_{14}}{\rightleftharpoons} B—Hm—B' \tag{14}$$

$$O_2 + B—Hm \overset{K_{15}}{\rightleftharpoons} B—Hm—O_2 \tag{15}$$

$$B—Hm—O_2 + B—Hm \overset{K_{16}}{\longrightarrow} [B—Hm—OO—Hm—B] \tag{16}$$

$$B—Hm—OO—Hm—B \overset{K_{17}}{\underset{\text{fast}}{\longrightarrow}} 2 B—Hm—O· \tag{17}$$

$$B—Hm—O· \overset{\text{fast}}{\longrightarrow} \text{products} \tag{18}$$

The mechanism will be assumed to be rate-limiting formation of the peroxide (reation 16) followed by fast steps leading to product. This is kinetically equivalent to fast reversible reaction (16) followed by a slower reaction (17). With the assumption that reaction 16 is rate-limiting the rate is:

$$\frac{d\,Hm^+}{dt} = R = k_{16}(B—Hm)\,(B—Hm—O_2) \tag{19}$$

Without added base, the concentrations of B—Hm and B—Hm—O_2 can be written in terms of O_2 concentration and total Hm^{II} concentration, $\Sigma\,Hm^{II} = HM$

$$R = k_{14}\left(\frac{1}{1 + K_{15}O_2}\right)\left(\frac{K_{15}O_2}{1 + K_{15}O_2}\right)HM^2 = \frac{k_{16}HM^2K_{15}(O_2)}{[1 + K_{15}(O_2)]^2} \tag{20}$$

At low oxygen concentration the rate becomes

$$R_{\text{low } O_2} = k_{15}K_{16}O_2HM^2 \tag{21}$$

At high oxygen concentration where the heme is in the oxyheme form and $K_{13}O_2 \gg 1$, the rate is given by:

$$R_{\text{high } O_2} = \frac{k_{16}HM^2}{K_{15}(O_2)} \tag{22}$$

This is in accord with our observations and affords an approximate value of k_{16} (or its equivalent $K_{16}k_{17}$). From the observed $k_{obsd} = 25$ M^{-1} s^{-1} and $K_{15} \cong 10^6$ M^{-1}, and oxygen concentration of 10^{-3} M (1 atm), the value of k_{16} is estimated to be about 2×10^4 M^{-1} s^{-1}. This large rate constant clearly shows that oxidation is too rapid in solution to allow high concentrations of oxyheme to be achieved unless very high concentrations of oxygen are used. However, it also indicates that, at low heme concentration and high oxygen concentration, it should be possible to retard oxidation sufficiently to allow physical measurements to be made on solutions.

If, as in the case of the studies of Cohen and Caughey, an external base is added, we can, for simplicity, treat their system as if a single external base is added to the system just described. This ignores the possibility of oxidation of four-coordinate heme (*i. e.*, ignores eq. 23)

$$Hm + O_2 \xrightarrow{k_{13}} Hm^+ + O_2^- \cdot \tag{23}$$

which, according to our observations, is a very fast process. By adding the equilibrium (14) to the system just discussed we can derive a rate expression in both oxygen and in the external base B,

$$\frac{d\,Hm^+}{dt} = R = \frac{k_{16}K_{15}HM^2(O_2)}{(K_{14}B' + K_{15}O_2)^2} \tag{24}$$

At low oxygen concentration where $K_{14}B' \gg K_{O_2}$, this rate becomes

$$R_{\text{low } O_2} = \frac{k_{16}K_{15}HM^2(O_2)}{K_{14}^2(B')^2} \tag{25}$$

the form observed at high pyridine and low oxygen concentration[9a].

At high oxygen concentrations where oxygenation is complete ($K_{O_2} \gg K_{12}B$) the rate should become

$$R_{\text{high } O_2} = \frac{k_{16}HM^2}{K_{15}O_2} \tag{26}$$

However, this situation was not realized in the experiments of Cohen and Caughey. As the oxygen pressure was raised and the pyridine concentration lowered the reaction tended toward eq. 25 in that the dependence upon oxygen and pyridine decreased.

Taken together these studies document the bimolecular peroxide mechanism for heme oxidation. The successful prevention of bimolecular autoxidation by introducing sufficient steric hindrance into the heme to prevent the peroxide formation[13,14] further corroborates this mechanism.

The observation of autoxidation which is first-order in heme at low pyridine concentrations[8,9a] is not explained by these mechanisms and therefore might be attributed to slow dissociation of the dipyridyl compound followed by fast oxidation.

$$\text{B—Hm—B'} \xrightarrow{\text{slow}} \text{B—Hm} \xrightarrow[\text{(O}_2\text{)}]{\text{fast}} \text{B—Hm}^+ \tag{27}$$
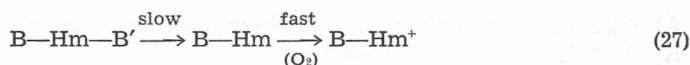
However, the rate of this reaction would have to be identical to the fastest rate of reaction of carbon monoxide observable with B—Hm—B', and such rates are orders of magnitude faster than the rates observed by Cohen and Caughey at room temperature. It seems more likely that there is, beside the bimolecular mechanism, a first-order process such as that suggested by Wang[8].

Protection of Hemes by Carbon Monoxide Complexation

Equation 24 suggests a means of stabilizing heme-oxygen mixtures and at the same time studying the heme-oxygen interaction. If the external base B' is carbon monoxide then the rate of autoxidation is given by eq. 25.

$$\frac{d\,Hm^+}{dt} = \frac{k_{16}K_{15}HM^2(O_2)}{(K_{14}CO + K_{15}O_2)^2} \tag{28}$$

This becomes eq. 26 when $K_{14} \gg K_{15}O_2$ and since $K_{14} \geq 10^7$ and $k_{15} \cong 10^6$, this situation is easily achieved[14].

$$\left(\frac{d\,Hm^+}{dt}\right)_{\text{high CO}} = \frac{k_{16}K_{15}HM^2(O_2)}{K_{14}^2(CO)^2} \tag{29}$$

Because the rate increases with the first power of oxygen concentration and the inverse second power of carbon monoxide concentration, the rate is decreased as the total pressure of a CO/O$_2$ mixture is increased. When the carbon monoxide is flashed off the heme, this system reverts temporarily to that described by eq. 20, or, at high O$_2$, to eq. 22.[11e] However, no oxidation occurs as long as the rate of return from B—Hm—O$_2$ to B—Hm—CO is much faster than that of eq. 22. This can be attained by increasing both the carbon monoxide concentration and the oxygen concentration. This approach has been used to study the kinetics of oxygenation of simple hemes for the first time[11a,f]. By using high pressures (> 1 atm) of carbon monoxide and oxygen, it should be possible to study the equilibrium oxygenation of simple hemes in solution.

Solvent Effects

The rates of oxidation in methylene chloride are much faster than those in dimethyl formamide even though both are second-order in heme and both presumably react by the same mechanism. Because the spectrum of the deoxyheme is the same in these two solvents, the differences cannot be attributed to the ligation of DMF (acting as B in eq. 27). We have previously shown that the equilibrium (15) is shifted to the right by polar solvents[11d,e], due to the polar nature of the Fe$^+$—OO$^-$ bond[11d,e]. Similarly, because reaction 16 reduces the polarity, it should be retarded by polar solvents. Therefore, the oxidation rate is retarded by such polar solvent as DMF or DMF-water.

This does not explain the rapid oxidation in water or methanol-water, or the rapid oxidation in the presence of weak acids. These accelerated oxidations are probably due to the polar oxidation discussed by Wang[8] and suggested for hemoglobin by Wallace, Maxwell and Caughey[9c] (eq. 3).

On the other hand, autoxidation of hemes such as 2 in benzene is very rapid at room temperature[11d]. Under these conditions the heme is only partially

oxygenated, and because the rate is proportional to the product of the concentrations of Hm and Hm—O_2 (eq. 16), it is maximized by having both low oxygenation and no external base for hexacoordination. In this case substitution of pyridine for imidazole and benzene for DMF have the effect of lowering K_{15}, with the result that eq. 21 takes the place of eq. 22 and rapid oxidation must ensue at high oxygen concentration.

While the solvent effects upon the bimolecular mechanism for oxidation are understandable in terms of K_{15} and k_{16}, the occurrence of a second ionic autoxidation mechanism makes the rates of autoxidation in hydroxylic solvents difficult to predict.

A third mechanism for autoxidation of hemes must also be considered (eq. 23). The four-coordinate heme reaction should become important at low base concentration or in hemoproteins, in which the proximal base-heme bond is very strained[15] and tends to break[11c,16] (K_{13} is small). As a result of the low oxidation potential of four-coordinate iron(II) porphyrins the electron-transfer process (either inner or outer sphere)

$$\diagdown \diagup \qquad \diagdown \diagup$$
$$\underset{\diagup \diagdown}{Fe} + O_2 \rightleftharpoons \underset{\diagup \diagdown}{Fe^+} + O_2^{-\cdot} \qquad (29)$$

should be very fast. This mechanism results in a rate expression

$$\frac{d\,Hm^+}{dt} = \frac{K_{21}HM(O_2)}{B^2K_{11}K_{12}+B(O_2)K_{11}K_{13}+BK_{11}+1} \qquad (30)$$

This mechanism could explain the observed first-order reaction (eq. 6) at low base concentration but not the second-order behavior at high concentration or the inverse dependence upon oxygen. At present, it is not clear whether the first-order oxidation (eq. 6) is due to the direct electron transfer from Hm (eq. 23) or from B—Hm (eq. 3).

Relationship to Hemoglobin and Myoglobin Autoxidations

It is not possible for two myoglobin molecules to come together in the manner of eq. 8. Yet myoglobin is autoxidized more rapidly at low pressures of oxygen[8] just as is our synthetic myoglobin site 1. This similarity could be fortuitous and result from, e. g., the competition of oxygen with some electron carrier for the iron site in myoglobin. Alternatively, if oxyheme does not dissociate from globin whereas deoxyheme dissociates at a measurable rate, then oxygen could protect against such dissociation. The dissociated heme is known to oxidize very rapidly. Thus, it is possible that the heme dissociates, oxidizes, and reassociates, although the oxidation of heme proteins in crystals seems to favor the first mechanism.
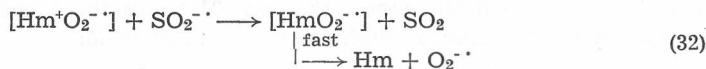
The oxygen pulse reaction rates from Table II indicate a linear dependence upon dithionite ion described by eq. 31.

$$\frac{d\,Hm\,O_2}{dt} = k_a\,(HmO_2) + k_b\,(HmO_2\,(SO_2^-)) \qquad (31)$$

where $k_a = 115$ s^{-1} should correspond to the oxygen off rate for 2—O_2 and $k_b = 3.6 \times 10^4$ M^{-1} s^{-1} to the bimolecular reaction of HmO_2 with dithionite

ion. The $k_a = 115$ s^{-1} is in reasonable agreement with a $k_{off} = 90$ s^{-1} observed for $1—O_2$ in the same solvent by flash photolysis methods.

The bimolecular reaction might proceed by either the mechanism indicated by eq. 32 or 33.

$$[Hm^+O_2^{-\cdot}] + SO_2^{-\cdot} \longrightarrow [HmO_2^{-\cdot}] + SO_2$$

$$\Big|\text{ fast} \tag{32}$$

$$\Big|\longrightarrow Hm + O_2^{-\cdot}$$

or

$$Hm—O—O + SO_2^{-\cdot} \rightarrow Hm + [\cdot OOSO_2] \rightarrow \text{product} \tag{33}$$

The similarities of rate constants for reaction of dithionite with the hemin 1^+ (eq. 12 with $k_2 = 3 \times 10^4$ M^{-1} s^{-1}) and with $2—O_2$ (eq. 31, $k_b = 3.6 \times 10^4$ M^{-1} s^{-1}) suggest that the electron-transfer process (32) occurs. If this is true, it constitutes interesting chemical evidence for the Fe^{III} state[18] of oxyheme compounds.

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DISCUSSION

L. K. Hanson:
Extended Hückel calculations, which I have performed on imidazole and pyridine ferrous heme CO and O_2 complexes, indicate that imidazole is more »electron-donating« (there is a charge density shift toward the CO/O_2).

T. G. Traylor:

This is in satisfying agreement with the results we obtained with oxygen, but we seem to have found a lesser difference between pyridine and imidazole in CO binding.

W. Scheler:

(1) Have you measured the oxidation-reduction potential? It would be interesting with respect to the low autooxidation rate. (2) Have you tried to crystallize your compounds?

T. G. Traylor:

(1) We have not as yet made any oxidation-reduction potential measurements. However, Harburg and coworkers have made extensive studies of cytochrome c octapeptide, which resembles our model. Unfortunately, the potentials have not been measured in different solvents, where large differences in stability can be seen. This should be done, however. (2) Our compounds have not been crystallized despite some efforts. The tetraphenylporphyrins are much more suitable for crystallization.

F. Jung:

Do your complexes show peroxidatic or catalatic activity?

T. G. Traylor:

We have not studied the peroxidase and catalase activities. However, a cytochrome c undecapeptide, which is similar to our models, is called »microperoxidase« because it also shows this activity.

I. C. Gunsalus:

Do you accept the Mössbauer data of Leo Lang on oxymyoglobin and Peter Debrunner on cytochrome $P450 \cdot O_2$ as showing ferric iron character?

T. G. Traylor:

The solvent effects upon oxygen binding we got, are parallel to those obtained by Stynes and Ibus with cobalt model compounds. Thus, I would accept the iron^{III}—O_2^-· formulation, with the reservation that dissociation of this complex in order to give free O_2^-· is slow even in aqueous solutions.

R. Austin:

Wouldn't binding of oxygen to the heme, and concomitant loss of the triplet ground state of oxygen, make the O_2 more reactive towards chemical attack?

T. G. Traylor:

Let me qualify my statement that the oxyheme is less active than triplet oxygen because the oxyheme isn't triplet. The oxyheme isn't a state of oxygen at all, but an oxygen compound just as *e. g.* a dioxetane is. Having no unpaired electrons on oxygen it does not react rapidly with radicals such as SO_2^-· *etc.*, as would triplet dioxygen or peroxy radicals. On the other hand, the oxyheme should be more basic than dioxygen.

SAŽETAK

Kinetika i mehanizmi oksidacije hemoproteinskih modelnih spojeva

C. K. Chang, D. Powell i T. G. Traylor

Proučavana je kinetika reakcije kompleksa oksihema s ionom ditionita i s deoksihemom. Budući da su brzine autooksidacije kompleksa oksihema bile obrnuto proporcionalne tlaku kisika a proporcionalne kvadratu ukupne koncentracije hema, zaključuje se da se reakcija odvija preko stvaranja kompleksa hem-OO-hem, kako su to predložili Cohen i Caughey. Direktna reakcija ditionit-iona s kompleksima oksihema slaže se s formulacijom veze željezo-kisik kao $Fe^+O_2^-$·.

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