(2 H, m), 6.92 (2 H, m). Anal. Calcd for $C_{17}H_{26}N_4O_2S$: C, 58.3; H, 7.5; N, 16.0; S, 9.2. Found: C, 58.0; H, 7.6; N, 15.7; S, 9.4.

2-(Tetrahydropyran-2-ylthio)-1-methoxy-2-methyl-1,1-bis(1-methylimidazol-2-yl)propane (V). Sodium hydride (6.5 g of a 50% mineral oil dispersion, 0.14 mol) was washed twice with hexane and then suspended in dry DMF (100 mL) under nitrogen. The vigorously stirred mixture was placed in a cooling bath kept at 15 °C and crude IV (47.0 g, 0.13 mol) in dry DMF (200 mL) added over 15 min. When the gas evolution had ceased (30 min), the mixture was cooled to 5 °C and methyl iodide (19.0 g, 0.13 mol) added at such a rate that the reaction temperature did not exceed 10 °C. The mixture was stirred for 30 min with cooling and then for 1 h at room temperature and then quenched by the addition of water (2.75 L) containing NH_4Cl (15 g). The mixture was extracted with benzene, and the combined organic layers were washed with water and dried over $MgSO_4$. Removal of the benzene under reduced pressure left a residue which solidified on standing. The residue was dissolved in boiling cyclohexane (500 mL) and the product V obtained by the addition of hexane (250 mL). A second crop was obtained by concentrating the filtrate. The total yield of V, a white crystalline solid, was 32.0 g (66%); mp 125–126 °C. ^1H NMR: δ 1.2–1.8 (6 H, m), 1.90 (3 H, s), 2.0 (3 H, s), 3.16 (6 H, s), 3.3–4.5 (3 H, m), 3.54 (3 H, br s). Anal. Calcd for $C_{18}H_{28}N_4O_2S$: C, 59.3; H, 7.7; N, 15.4; S, 8.8. Found: C, 59.6; H, 7.8; N, 15.5; S, 9.0.

1-Methoxy-2-methyl-1,1-bis(1-methylimidazol-2-yl)-2-propanethiol (VI). A solution of V (20.0 g) in CF_3CO_2H (100 mL) was refluxed for 1 h. Most of the CF_3CO_2H was removed under reduced pressure and the residue, a dark brown oil, was shaken with benzene (400 mL) and water (3 × 700 mL) after which all of the oil dissolved. The combined aqueous layers were extracted with benzene and the extracts discarded. The aqueous phase was neutralized by the addition of solid $NaHCO_3$ and thoroughly extracted with benzene. The combined extracts were dried over $MgSO_4$, and the solvent was removed under reduced pressure. The residue was dissolved in boiling cyclohexane (500 mL) and the solution allowed to cool to room temperature then rapidly filtered through Celite. The filtrate was concentrated to 150 mL, and upon cooling a white precipitate appeared. Precipitation was completed by the slow addition of hexane (300 mL) to give VI, a white crystalline solid (12 g, 78%, mp 122–124 °C). ^1H NMR: δ 1.75 (6 H, s), 3.34 (9 H, s), 3.60 (1 H, br s), 6.75 (2 H, d, J = 2 Hz), 6.93 (2 H, d, J = 2 Hz). Anal. Calcd for $C_{13}H_{20}N_4OS$: C, 55.7; H, 7.2; N, 20.0; S, 11.4. Found: C, 55.8; H, 7.3; N, 19.7; S, 11.5.

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Registry No. I, 2365-48-2; II, 76024-79-8; III, 76024-80-1; IV, 76024-81-2; V, 76024-82-3; VI, 76036-51-6; IX, 38634-58-1; X, 76024-83-4; $CH_3OCH_2SC(CH_3)_2CN$, 76024-84-5; $(H_2NCH_2C(CH_3)_2S-)_2 \cdot H_2SO_4$, 76024-85-6; $(H_2NCH_2C(CH_3)_2S-)_2 \cdot HClO_4$, 76024-86-7; $CH_3OCH_2SC(CH_3)_2CH_2NH_2$, 76024-87-8; CH_3COSK, 10387-40-3; CH_3OCH_2Cl, 107-30-2; $ClCH_2CN$, 107-14-2; MeI, 74-88-4; DHP, 110-87-2; *N*-methylimidazole, 616-47-7; $[Cu(VI)_2]^0$, 76036-74-3; $[Cu(Me_4\text{-cystam})CH_3CN](ClO_4)_2$, 76036-73-2; $[Cu(Me_4\text{-cystam})(N\text{-methylimidazole})](ClO_4)_2$, 76036-71-0.

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Chlorite Ion Oxidation of the Iron(III) Complex of Deuteroporphyrin IX

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The extent and rate of oxidation of the iron(III) complex of deuteroporphyrin IX by sodium chlorite have been followed via stopped-flow spectrophotometric measurement of the decrease in absorbance in the Soret-band region (384 nm) of the heme spectrum. The reaction results in formation of one or more reaction intermediates (an "intermediate state") containing iron in oxidation state >Fe(III) which play(s) a central role in the (peroxidatic) catalytic activity of the heme. At pH 6.5–7.0 the apparent molar equivalency [heme Fe(III)]:$[ClO_2^-] \simeq 4:1$, but this ratio as well as the rate of intermediate formation decreases with increasing basicity. It is speculated that the extent and rate of intermediate production under given conditions are determined by the relative importance of (1) formation of the intermediate state from heme and oxidant and (2) its subsequent collapse via catalytic or other pathways with accompanying heme regeneration. The apparent stoichiometry at lower pH corresponds to a one-electron oxidation of each of four heme Fe(III) units to Fe(IV) for the transformation of $ClO_2^- \rightarrow Cl^-$, suggesting an intermediate state analogous to peroxidase Compound II species. The possibility of a rate-limiting oxidation of heme Fe(III) to a Compound I analogue, regarded formally as an Fe(V) species, with subsequent comproportionation, Fe(V) + Fe(III) → 2Fe(IV), also is considered. Heme oxidation by hypochlorite ion is measurably faster than oxidation by ClO_2^-, which suggests the possibility of OCl^- as a reactive intermediate in the chlorite reaction. The absorption spectrum of the intermediate state is analogous to that obtained via oxidation of heme with selected peroxobenzoic acids. Comparable kinetic parameters obtained for the oxidation of iodide ion and phenol by intermediate states derived from chlorite and peroxo substrates further indicate that the same oxidized form of heme is obtained via reaction with both types of oxidant.

Protein-free hemes have been widely studied as models of heme-containing enzymes including catalase and various peroxidases which mediate the peroxide oxidation of selected substrates.[1-4] Investigations include those of the kinetics and mechanism of their reaction with peroxo substrates to form intermediate species which play a central role in the catalytic activity of the heme.[5-7] Such intermediates are presumed to represent oxidized forms of heme where the iron center exists in oxidation state >Fe(III) and, indeed, for many years it has been assumed that the principal species so obtained is a product of a two-electron oxidation process and, therefore, an analogue of species derived from catalase and peroxidase enzymes denoted as Compounds I.[8-10]

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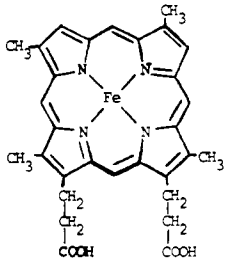
Relative to such investigations, much attention has been devoted to the reaction of the iron(III) complex of deuteroporphyrin IX, deuteroferriheme (I below), with hydrogen



I

peroxide; however, this substrate is highly susceptible to heme-catalyzed decomposition, a process which is thought to involve substrate oxidation by the corresponding heme–peroxo intermediate. This catalase-like or *catalatic* action of deuteroferriheme on H_2O_2 has complicated efforts to study the stoichiometry of intermediate formation in this system. Other substrates such as selected peroxo carboxylic acids seem more amenable for such study since they react with heme to produce peroxidatically active intermediate species at rates which are rapid compared to the rate of subsequent heme regeneration via catalatic or other pathways. In this regard, reactions of deuteroferriheme with various substituted peroxobenzoic acids have been carried out in which the apparent "extent of reaction" has been followed by determining the change in optical density of buffered heme solutions brought about by "titrimetric" stopped-flow addition of various amounts of peroxoacid. Somewhat unexpectedly, such studies have shown 1 mol of peroxo acid to be formally equivalent to two heme Fe(III) species.[11] Since each mole of peroxo acid contains 2 oxidizing equiv, this corresponds to an "intermediate state" in which iron may be considered to exist formally as Fe(IV), analogous to peroxidase–Compound II species.[12] Since it has long been believed that such enzyme-derived species are obtained not from *direct* hemoprotein oxidation but rather by reduction of an initially formed two-electron oxidation product (a Compound I intermediate), it has been speculated that the mechanism of intermediate formation from heme and peroxo acid involved rapid reaction of free heme Fe(III) with an initially formed heme–Compound I analogue containing iron as Fe(V).[11]

In a continuation of studies of hemes as peroxidase analogues and, specifically, of the kinetics and stoichiometry of intermediate formation, we have investigated the reaction of deuteroferriheme with chlorite ion as a function of pH. Since ClO_2^- is a four-electron oxidant, it was anticipated from a knowledge of the heme–peroxo acid stoichiometry that a molar equivalency Fe(III):ClO_2^- ≈ 4:1 might be observed. Added interest to such a study has been provided by reports of the reaction of the enzyme horseradish peroxidase (HRP) with chlorite ion,[13-15] including recent studies of Hewson and Hager,[16] who have concluded that a direct oxidation of HRP by chlorite to a one-electron oxidation product, i.e., Compound II, may occur without prior intervention of the two-electron-oxidized Compound I entity.

Experimental Section

Materials. Triply crystallized hemin was obtained from Nutritional Biochemicals Corp. and converted to deuteroferriheme by the resorcinol melt method.[17] The recrystallized product was characterized as the pyridine hemochrome derivative as previously described.[7,17,18] Sodium chlorite (MCB granular reagent) was of ≥99% purity as determined by iodometric analysis. *m*-Chloroperoxobenzoic acid was obtained from Pfalz and Bauer in ~85% purity, the only significant impurity being the parent carboxylic acid. Pure samples of peroxo acid were prepared by recrystallization from 3:1 petroleum ether–Et_2O. Titration of aqueous stock solutions with Ce(IV) in the presence of ferroin showed negligible hydrogen peroxide production over a period of 24 h. Both commercial grade and purified material were kinetically indistinguishable in all investigations. Sodium hypochlorite (Mallinckrodt) was obtained as an alkaline stabilized 5% aqueous solution. Approximately 10^{-3} M stock soutions were prepared by dilution and analyzed iodometrically for OCl^-. Since decomposition of hypochlorite is pH dependent, subsequent dilutions of the stock solution were performed with use of appropriate buffer solutions. Carbonate and phosphate buffer components were of analytical reagent grade. All solutions were prepared with use of deionized water which was subsequently distilled or passed through a Barnstead mixed-bed ion-exchange column (conductivity <0.01 ppm as NaCl).

Methods. Spectra were obtained with use of Beckman Model 5230 and Gilford 250 visible–UV spectrophotometers. Stoichiometry and rate studies were carried out at 25 °C with a Durrum-Gibson D-110 stopped-flow spectrophotometer in conjunction with a Tektronix oscilloscope. Spectrophotometric "titrations" involved measuring the maximum decrease in absorbance, ΔA_{max}, accompanying the stopped-flow mixing of buffered heme solutions with aqueous solutions containing varying amounts of $NaClO_2$, the initial reference absorbance reading having been obtained from a mixture of the given heme solution with water. The stopped-flow trace from initial absorbance at t_0 to the time of attainment of ΔA_{max} also allowed calculation of kinetic parameters for conversion of heme to the one or more peroxidatically active species to which we refer as the "intermediate state". In subsequent discussions, A_0 refers to the absorbance at the beginning of reaction, A_t, to that of the solution at any reaction time, t, and A_∞ to that of the solution at t_∞, i.e., when the maximum decrease in optical density has been obtained. In some cases, "initial rates" of reaction were followed by observing the nearly linear change in absorbance with time immediately following the stopped-flow mixture of reagents. Typical concentration ranges for stopped-flow studies were 7–50 μM deuteroferriheme and 0.5–10^3 μM sodium chlorite and *m*-chloroperoxobenzoic acid. Ionic strengths were maintained at 0.1 M by addition of reagent grade NaCl to appropriate buffer solutions.

Spectrum of the Intermediate State. Absorbance values obtained from stopped-flow mixtures of heme and water relative to water as a reference at various wavelengths allowed point by point determination of the spectra of given heme solutions. The spectrum of the intermediate state was obtained by mixing heme with sufficient chlorite or peroxo acid to give the maximum change in absorbance, ΔA_{max}.

Peroxidatic Oxidation of KI and Phenol. The oxidation of iodide ion by the heme–chlorite-derived intermediate state was followed by use of stopped-flow techniques. The intermediate state was preformed by mixing a buffered deuteroferriheme solution with $NaClO_2$ in desired proportions and allowing an appropriate time interval for attainment of ΔA_{max}. This solution was then rapidly introduced into a stopped-flow drive syringe following which it was mixed with an aqueous solution of KI. The oxidation reaction was followed by measuring the increase in absorbance at the Soret-band maximum at 384 nm. Experiments were performed at a fixed initial heme concentration of 4 μM from pH 6.9 to 10.3. At pH 6.9 the ratio of initial concentration [heme]:[ClO_2^-] was set equal to 4:1 whereas, at pH 10.3, this ratio was lowered to 0.5:1 to produce maximum yields of intermediate. Potassium iodide concentrations in the range 2.5–12.5 mM were used

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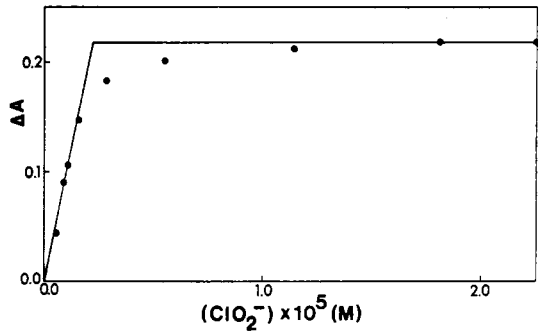

Figure 1. Stopped-flow spectrophotometric "titration" of deuteroferriheme with $NaClO_2$: λ = 354 nm; T = 25 °C; pH 7.10; μ = 0.1 M; $[heme]_0$ = 9.2 μM.

Table I. pH Dependence of the Heme Fe(III):ClO_2^- Ratio at ΔA_{max}

pH	λ, nm	[heme], μM	heme Fe(III): ClO_2^-
6.77	369	5.1	3.81
7.10	354	9.2	4.08
7.36	360	12.7, 17.7	2.96, 2.97
7.76	362	12.5, 15.6	2.27, 2.20
8.23	369	5.1, 12.8	1.38, 1.78
8.40	369	5.0, 12.4	1.57, 1.42
8.69	369	12.5, 15.6	1.40, 1.34
9.13	369	12.5	0.80

to enable us to study the heme regeneration under pseudo-first-order conditions. A similar procedure was used to study the oxidation of phenol.

Results

As in reactions of deuteroferriheme with peroxo substrates, the heme–chlorite reaction is accompanied by a decrease in optical density in the Soret region of the heme spectrum attributed to the oxidation of heme to one or more reaction intermediates. After attainment of maximum absorption change, ΔA_{max}, a slow regeneration of optical density occurs, signifying heme regeneration. The rate of regeneration is complex, depending on pH and initial concentrations of reactants. A stopped-flow spectrophotometric heme–ClO_2^- "titration" curve obtained at pH 7.1 is shown in Figure 1 and yields a molar equivalency ratio [heme Fe(III)]:$[ClO_2^-]$ = (4.0 ± 0.2):1. However, this "apparent stoichiometry" of intermediate formation, determined as the relative proportions of reactive species leading to maximum decrease in optical density, is dependent on hydrogen ion concentration and decreases markedly with increasing pH. This is illustrated by data in Table I.

Point spectra resulting from stopped-flow mixtures of heme with sodium chlorite and with *m*-chloroperoxobenzoic acid at pH 6.78 are presented in Figure 2. For each of these studies, the oxidant was in sufficient concentration to produce plateau absorbance readings such as shown in Figure 1 and, presumably, a quantitative conversion of heme to reactive intermediate(s). Within experimental error, the corresponding intermediate states appear to be spectrally identical. On the other hand, spectral results at pH 9.18 are quite different in that the spectrum obtained from a comparable mixture of deuteroferriheme and chlorite appears to show characteristics of both the original heme spectrum and that of the peroxo-acid-derived intermediate, suggesting only partial chlorite oxidation of heme to intermediate at the higher pH.

Some results of a study of the peroxidase-like activity of heme are given in Figure 3 which shows the correspondence of rates, and essential pH independence of rates, of iodide oxidation by intermediate(s) derived from the oxidation of heme by sodium chlorite and hydrogen peroxide, respectively.[19]

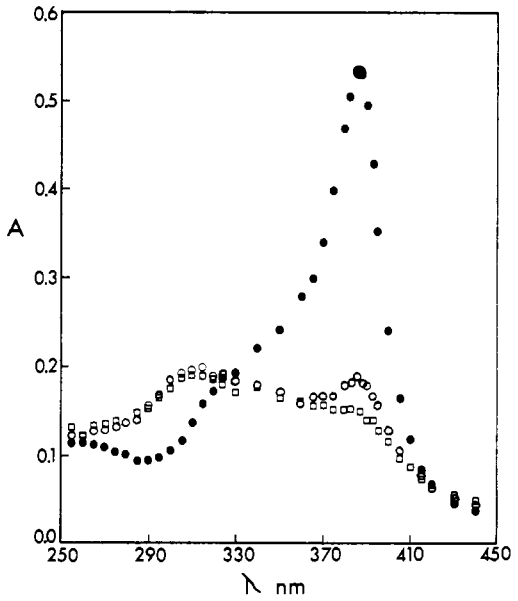

Figure 2. Absorption spectra obtained via stopped-flow spectrophotometric studies (pH 6.78; μ = 0.1 M): (●) 5.1 μM deuteroferriheme; (○) intermediate state from 5.1 μM heme + 2.3 μM $NaClO_2$; (□) intermediate state from 5.1 μM heme + 4.2 μM *m*-chloroperoxobenzoic acid.

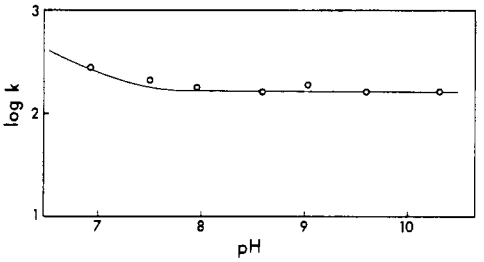

Figure 3. Rate of oxidation of iodide as function of pH (μ = 0.1 M; T = 25 °C; $[I^-]$ = 2.5–12.5 mM): (○) oxidation by intermediate state derived from heme [4.0 μM] and $NaClO_2$; (—) data from oxidation by intermediate state derived from heme and H_2O_2;[20] $k = k_{obsd}/[I^-]$; $k_{obsd} \equiv -d \ln (A_\infty - A_t)/dt$.

Although phenol oxidation displays pronounced pH and heme concentration dependence[21] and, therefore, proceeds mechanistically in a different manner from iodide oxidation, comparable rates of oxidation of phenol at fixed pH by the two intermediate states are also observed. Thus, in addition to a spectral correspondence, such intermediate states display a kinetic equivalence in terms of selected peroxidatic activity.

The kinetic pattern for absorbance change accompanying the heme–chlorite reaction is formally similar to that observed for deuteroferriheme oxidation by hydrogen peroxide and resembles a modified Michaelis–Menten scheme.[7] Thus, at relatively high $[ClO_2^-]$:[heme] ratios, the approach to ΔA_{max} is first order as evidenced by linearity of a plot of $\ln (A_t - A_\infty)$ vs. time over a period exceeding 2 half-lives with the chlorite ion concentration dependence of the resultant pseudo-first-order rate constant, k_{obsd}, showing typical saturation kinetics. Calculated second-order constants derived from the slopes of k_{obsd} vs. $[ClO_2^-]$ curves at low substrate concentrations, where first-order dependence of rate on chlorite ion is observed, show a heme concentration dependence. Increasing the initial heme

(19) Intermediate states obtained by reaction of deuteroferriheme with peroxo acids, hydrogen peroxide, and *tert*-butyl hydroperoxide have been reported to be kinetically equivalent in the oxidation of iodide ion, leading to the conclusion that the same oxidized form of deuteroferriheme exists in each system.[20]

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Table II. Heme Concentration Dependence of the Rate of Formation of Intermediate State (T = 25 °C)

pH	[heme], μM	$10^{-3}k_2$,[a] M^{-1} s^{-1}	α[b]
7.36	5.1	35	0.297
	8.9	31	0.235
	12.7	22	0.201
7.76	8.7	13	0.157
	12.5	10	0.133
	15.6	8.9	0.120
8.40	5.0	5.2	0.103
	12.4	3.0	0.066
8.69	4.7	3.1	0.077
	12.5	1.6	0.048
	15.6	1.3	0.043

[a] $k_2 = k_{obsd}/[ClO_2^-]$; $k_{obsd} \equiv -d \ln (A_t - A_\infty)/dt$. [b] α = fraction of deuteroferriheme present in monomer form.[22]

Table III. Rate of Attainment of Intermediate State via Oxidation of Deuteroferriheme by Sodium Chlorite (T = 25 °C)

pH	$10^{-4}k'$,[a] M^{-1} s^{-1}	pH	$10^{-4}k'$,[a] M^{-1} s^{-1}
6.77	18[b]	8.23	3.5[c]
7.08	18	8.40	4.8[c]
7.10	15[c]	8.69	3.5[b]
7.36	12[b]	9.13	1.7[c]
7.76	7.7[b]	10.1	1.4

[a] $k' = k_2/\alpha$; $k_2 \equiv k_{obsd}/[ClO_2^-]$; $k_{obsd} \equiv -d \ln (A_t - A_\infty)/dt$; α ≡ fraction of deuteroferriheme present in monomeric form. [b] Average of three determinations. [c] Average of two determinations.

concentration results in a decrease in the observed second-order rate constant at fixed pH (Table II). Comparable results, obtained in studies of the kinetics of heme oxidation by H_2O_2,[6] have been attributed to the concentration dependence of the degree of heme dimerization in aqueous solution.[22] As in the heme–H_2O_2 system, the effect is eliminated by dividing each second-order constant by a term, α, which is dependent on both heme concentration and pH and which is interpreted as the fraction of stoichiometric free heme present in monomeric form. Resulting constants, denoted k', are given in Table III and reflect the second-order rate of approach to maximum absorbance change and, presumably, to attainment of maximum concentrations of reaction intermediate(s) via oxidation of heme monomer. Unlike the case of the reaction with selected peroxo substrates such as hydrogen peroxide and *m*-chloroperoxobenzoic acid, k' for heme oxidation by ClO_2^- increases with decreasing pH. A simple order in hydrogen ion does not emerge, however, since a plot of log k' vs. pH gives slope ≃ −0.4. A comparable fractional order in hydrogen ion also describes the pH dependence of the "initial rate" of reaction.

A consideration of the possibility of the formation and subsequent involvement of hypochlorite ion in the heme–chlorite reaction has led to a brief investigation of the kinetics of heme oxidation by OCl^-. Over the range pH 8.23–10.1, this reaction was observed to be measurably faster than that between heme and $NaClO_2$. For example, the observed second-order rate constant at pH 8.23 and 25 °C is 3.9×10^5 M^{-1} s^{-1} (3.2×10^6 M^{-1} s^{-1} with the assumption that heme monomer is the species oxidized). The corresponding parameters for heme oxidation by ClO_2^- are 4.3×10^3 and 3.5×10^4 M^{-1} s^{-1}. Interestingly, the rate of subsequent regeneration from the intermediate state formed in heme–OCl^- systems also is rapid compared to regeneration from the chlorite-derived intermediate, and both formation and regeneration are accelerated with increasing pH. Such regeneration is sufficiently rapid to prevent the demonstration of an integral stoichiometric relationship between heme Fe(III) and OCl^-. Although a 2:1 molar equivalency is predicted, the observed [Fe(III)]:[OCl^-] ratios range from 1.0 to 1.7 in the region pH 9.35–10.1.

Measurable differences are also observed between the chlorite-derived intermediate state and that obtained from heme oxidation by *m*-chloroperoxobenzoic acid with respect to rates of regeneration of optical density following attainment of ΔA_{max}. A mixture of 50 μM heme and 20 μM ClO_2^- in phosphate buffer (pH 6.8) yields, upon attainment of the minimum absorbance level, a solution of sufficiently stable optical density to permit determination of its absorption spectrum by conventional spectrophotometric scanning techniques. The resulting spectrum is in good agreement with the point spectrum obtained by stopped-flow methods. By contrast, regeneration of heme from a corresponding solution prepared from peroxo acid is too fast for comparable spectral analysis. Preliminary studies using chlorite, peroxo acid, and hypochlorite show the regeneration rate to be dependent upon the ratio of initial concentrations of heme to oxidant used in forming the intermediate state.

Discussion

The action of chlorite ion on deuteroferriheme leads to the formation of one or more hyperoxidized heme species which exhibit peroxidatic activity, and both spectrophotometric and kinetic data indicate this intermediate state to be identical with that formed when a peroxide is used as an oxidant. In consideration of the substrate concentration dependence of the rate of formation of this intermediate state, including conformation to saturation kinetics, a stoichiometric mechanism similar to that proposed for heme oxidation by hydrogen peroxide is suggested[7] (Scheme I). Here E_m denotes active free deuteroferriheme (presumed to be heme monomer), S denotes substrate (ClO_2^-), ES, the initial heme-substrate complex, ES′, the intermediate state, and P, reaction products accompanying heme regeneration.[23]

Scheme I

$$E_m + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} ES' \xrightarrow{k_r} E + P$$

As noted earlier, the heme–H_2O_2 and heme–ClO_2^- systems differ with respect to the direction of pH dependence of the rate of intermediate formation. For oxidation by ClO_2^-, the fractional-order dependence of the rate of reaction on hydrogen ion is not well understood, nor is it addressed in Scheme I. Since "initial rates" of reaction display this complex pH dependence, the effect is not due simply to partially compensating effects of coupled formation and regeneration reactions. General-acid catalysis may be involved in some manner perhaps comparable to what has been proposed to explain specific buffer effects in the heme–H_2O_2 system;[7] however, such a suggestion is tentative and the effect is undergoing further study.

More than one regenerative proces may be encompassed in k_r. Little is known of the mechanism of regeneration. In each of the systems studied, it appears to be kinetically dependent upon oxidant; thus, a catalatic decomposition of oxidant may represent a significant contribution to the regenerative process. In the heme–ClO_2^- system, such regeneration is slow relative to formation of the intermediate state, particularly at lower pH. This suggests an explanation for the pH dependence of the apparent stoichiometry.

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(23) As in the heme–H_2O_2 system, for the development of rate equations, ES′ is formally assumed to be a single kinetically significant species (see ref 7 for derivations).

At lower pH, presumably the formation reaction is sufficiently fast to provide quantitative conversion of heme to intermediate(s) before significant regeneration occurs and this gives rise to the observed stoichiometric relation Fe(III):ClO_2^- $\simeq$ 4:1. With increasing alkalinity, however, the relative importance of heme regeneration limits the accumulation of species comprising the intermediate state, producing an apparent decrease in stoichiometry.

Although details of the mechanism of formation and regeneration remain largely speculative due, in part, to uncertainities in the nature of the heme intermediate(s), the 4:1 molar equivalence of heme to ClO_2^- observed at pH $\sim$7 formally corresponds to a one-electron oxidation of each of four heme Fe(III) units to Fe(IV) for the transformation $ClO_2^- \rightarrow Cl^-$. A similar case is found in the reaction of heme with (two-electron-oxidizing) peroxo acids in which a [heme Fe(III)]:[oxidant] ratio $\simeq$ 2:1 is observed.[11] Such a one-electron oxidation of heme Fe(III) is suggestive of an intermediate state which is analogous to a peroxidase Compound II species.

As noted earlier, prevailing acceptance of the notion that Compound II species were found only via reduction of the primary two-electron-oxidation products of Fe(III), i.e., enzyme–Compounds I or their heme analogues, has prevented serious consideration of a direct Fe(III) $\rightarrow$ Fe(IV) oxidation process. Correspondingly, heme oxidation by peroxo acids has been considered in terms of a rate-limiting oxidation of Fe(III) to an Fe(V) Compound I analogue followed by its subsequent fast reaction with free Fe(III) to produce the stoichiometric equivalent of two Fe(IV) species.[11] The possibility of aggregation to a dinuclear compound containing iron in average oxidation state Fe(IV) has also been considered. Such a model has the attractiveness of allowing chlorite ion oxidation of heme to be viewed as proceeding via oxygen transfer to the Fe(III) center (Scheme II), with each $Fe^{V}O$ species undergoing rapid reaction with Fe(III) to produce two iron(IV) centers to accommodate the stoichiometry (a "ferryl" structure has been proposed as a possible configuration for Compound I species).[24-27] Intermediacy of OCl^- is consistent with kinetic observations since, as noted above, heme oxidation by sodium hypochlorite is measurably faster than by $NaClO_2$. By contrast, heme oxidation by hydrogen peroxide is slower than the heme–chlorite reaction which precludes any mechanism for the latter process invoking intermediacy of H_2O_2.[7]

Scheme II

$$Fe^{III} + ClO_2^- \rightarrow Fe^{V}O + OCl^- \quad \text{(slow)}$$

$$Fe^{III} + OCl^- \rightarrow Fe^{V}O + Cl^- \quad \text{(fast)}$$

An alternate free-radical scheme for oxidation of Fe(III) to Fe(IV) in four one-electron steps involving sequential intermediacy of OCl·, OCl^-, and Cl· can also be written (Scheme III).

Scheme III

$$\text{Fe(III)} + OClO^- \rightarrow \text{Fe(IV)} + OCl\cdot \quad \text{(rate limiting)}$$

$$\text{Fe(III)} + OCl\cdot \rightarrow \text{Fe(IV)} + OCl^-$$

$$\text{Fe(III)} + OCl^- \rightarrow \text{Fe(IV)} + Cl\cdot$$

$$\text{Fe(III)} + Cl\cdot \rightarrow \text{Fe(IV)} + Cl^-$$

An examination of the heme–chlorite system using ^{18}O-labeled chlorite or solvent may be helpful in establishing relative merits of such models. At present there is no kinetic evidence for such a distinction. There may be some convenience in viewing Fe(III) oxidation to occur via oxygen transfer in both peroxo acid and chlorite oxidations of heme; however, in view of recent findings of Hewson and Hager on the reactivity of horseradish peroxidase with chlorite ion, a direct one-electron oxidation of heme Fe(III) to an analogue of peroxidase Compound II without involvement of an initially formed two-electron oxidation product cannot be precluded.[16]

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