Influence of Ligand Modification on the Kinetics of the Reactions of Iron(III) Porphyrins with Hydrogen Peroxide in Aqueous Solutions

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The kinetics of the reactions of a series of iron(III) porphyrins with H_2O_2 to form oxidized, peroxidatically active intermediates have been studied, at 25 °C and ionic strength 0.1 mol dm^{-3}, by measuring the rates of coupled oxidation of hydroquinone (in some experiments ascorbic acid) under conditions where the reactions were of zero order with respect to reductant concentration. Changes in porphyrin structure were found to influence the reactivity of iron(III) porphyrins in two ways: (1) by affecting the degree of dimerization of the iron(III) porphyrin (rate constants for reaction of the monomeric species showed an inverse dependence on $[H^+]$; reaction of dimeric species was only observed for the iron(III) porphyrins having high dimerization constants and the rate constants were independent of pH); (2) to a much smaller extent by exerting a direct influence upon the rate constants for reaction of both monomeric and dimeric iron(III) porphyrin species with H_2O_2.

Iron(III) porphyrins (ferrihaems) display both catalase-like and peroxidase-like attributes in aqueous solution. Extensive studies of the decomposition of hydrogen peroxide catalysed by ferrihaems have shown that dimerization of ferrihaems has a major influence upon catalytic properties and that monomeric ferrihaems are much more effective catalysts than the corresponding dimers.[1-3] Thus modifications of the porphyrin ligand which influence ferrihaem dimerization (Table) have a marked effect upon the gross catalytic activities of these species. However, the influence on the specific catalytic activities of the different monomeric ferrihaems is much smaller and the kinetics of the reactions are closely similar for a range of iron(III) porphyrins.

Detailed studies of both the catalase- and peroxidase-like actions of the iron(III) complex of deuteroporphyrin IX (deuteroferrihaem) have shown that these reactions occur by an obligatory order mechanism in which the iron(III) porphyrin is first converted into an oxidized intermediate by reaction with H_2O_2.[4-6] This intermediate is then reduced back to the iron(III) state in oxidizing a range of donor substrates (including H_2O_2 itself). The mechanism thus bears a marked formal similarity to those for the catalases and peroxidases, in which the enzymes are first oxidized by H_2O_2 to form the Compound I intermediates, equations (1)—(3). For peroxidases

$$\text{Enzyme} + H_2O_2 \xrightarrow{k_1} \text{Compound I} + H_2O \quad (1)$$

$$\text{Compound I} + H_2O_2 \xrightarrow{k_4} \text{Enzyme} + O_2 + H_2O \quad (2)$$

$$\text{Compound I} + \text{Donor} \xrightarrow{k_5} \text{Enzyme} + \text{Oxidation Products} \quad (3)$$

in which reaction (2) does not occur studies of the kinetics of Compound I formation are straightforward. For catalases the situation is more complicated. Although direct measurement of k_1 might be possible, in principle, by studying the oxidation of a donor which reacted so efficiently with Compound I that the overall rate was determined only by the rate of reaction (1), no donor of adequate reactivity has been found and indirect methods have had to be employed.[7]

For deuteroferrihaem, Portsmouth and Beal [4] have demonstrated that hydroquinone is a very efficient donor for the oxidized intermediate, that experimental conditions are readily realized in which the steady-state oxidation of hydroquinone to benzoquinone is zero order with respect to hydroquinone concentration (*i.e.* intermediate formation is rate-limiting), and that, under these conditions, no irreversible destruction of the catalyst (by porphyrin oxidation) occurs. This 'peroxidatic' method permits a straightforward study of the reactions of H_2O_2 with iron(III) porphyrins to form the catalytic intermediates, in isolation from the overall 'catalatic' cycle. In the present work this technique has been applied to the reactions with H_2O_2 of the series of iron(III) porphyrins shown in the Table.

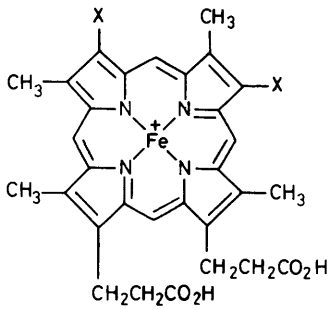
Experimental

Materials.—Protoferrihaem (pfh) was obtained as chromatographically pure 'haemin chloride' from Fluka, A.G., Switzerland. Deuteroferrihaem (dfh) was prepared from pfh by the resorcinol melt method.[8] Samples of coproferrihaem (cfh), mesoferrihaem (mfh), and haematoferrihaem (hfh) were gifts from Drs. S. B. Brown and H. Hatzikonstantinou. Their preparation and characterization has been described elsewhere.[9-11] Deuteroferrihaemdisulphonic acid (dds) was prepared by the method of Kolski and Plane.[12]

Hydrogen peroxide was obtained as a 35% (w/w) unstabilized aqueous solution (Laporte Industries Ltd., Widnes). More dilute solutions prepared from this material were assayed iodometrically or cerimetrically. Solutions of AnalaR grade quinol (hydroquinone) and L-ascorbic acid were prepared immediately before use. Buffer solutions were prepared from AnalaR Na_2HPO_4–KH_2PO_4 and Na_2CO_3–$NaHCO_3$ components and AnalaR sodium chloride was used for ionic strength adjustments (to 0.1 mol dm^{-3}).

Kinetic Measurements.—Experiments were carried out at 25 °C. Hydrogen peroxide solution and hydroquinone solution were introduced using micropipettes into a 2-cm cuvette mounted in a Unicam SP1800 spectrophotometer. Reaction was initiated by injecting ferrihaem solution (5 cm^3) in buffer into the cuvette from a calibrated syringe. Solutions of hfh were used within 15 min of preparation. The decrease in absorbance of hydroquinone was recorded (λ = 289 nm, $\varepsilon_{289} = 2.5 \times 10^3$ dm^3 mol^{-1} cm^{-1}). The initial concentration of hydroquinone was 140 μmol dm^{-3}, concentrations of hydrogen peroxide were in the range 100 μ mol dm^{-3}—5 mmol dm^{-3}, and ferrihaem concentrations in the range 0.5—20 μmol dm^{-3}. The variation of absorbance with time was linear in all

Table. Structures and dimerization constants of iron(III) porphyrins



Trivial name	Abbreviation	Porphyrin ligand	Substituent X	10^2K [a]	Ref.
Protoferrihaem [b]	pfh	Protoporphyrin IX	$CH{=}CH_2$	450	14
Mesoferrihaem	mfh	Mesoporphyrin IX	CH_2CH_3	6.92	10
Deuteroferrihaem	dfh	Deuteroporphyrin IX	H	3.40	13
Haematoferrihaem	hfh	Haematoporphyrin IX	$CH(OH)CH_3$	1.00	11
Deuteroferrihaemdisulphonic acid	dds	Deuteroporphyrin disulphonic acid IX	SO_3H	0.23	15
Coproferrihaem	cfh	Coproporphyrin III	$CH_2CH_2CO_2H$	0.21	9

[a] K = [Dimer][H^+]/[Monomer]^2 at 25 °C, ionic strength 0.1 mol dm^{-3}. [b] Systematic name: [3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionato(2−)]iron(III).

cases, confirming the zero-order nature of the reaction with respect to hydroquinone concentration. Check experiments at the Soret band maxima of the ferrihaem showed no catalyst destruction and the zero-order reaction rates, V_0, were calculated from the change in absorbance at λ = 289 nm. Corrections for the background oxidation rate were negligible in phosphate buffers (pH 6.5—8.05) but became unacceptably high at pH > 9 in carbonate buffers. Measurements with hydroquinone were therefore restricted to the phosphate range. In some experiments over a wider pH range ascorbic acid was used as reductant (initial concentration 28 μmol dm^{-3}, measurements being made at λ = 265 nm, $\varepsilon_{265} = 1.02 \times 10^4$ dm^3 mol^{-1} cm^{-1}). In these experiments background corrections were required at all pH values but were < 10% of the total oxidation rate.

Results and Discussion

For all systems the zero-order reaction rate, v_0, was directly proportional to $[H_2O_2]$ at constant pH and ferrihaem concentration, [T]. However, the variation of v_0 with [T] is complex since values of a rate constant k_T defined by equation (4)

$$v_0 = k_T[T][H_2O_2] \quad (4)$$

decreased systematically with increasing [T]. For different ferrihaems at constant [T] and pH the values of k_T increased approximately in the sequence of decreasing dimerization constants (Table) of the ferrihaems. This behaviour suggests that monomeric and dimeric ferrihaems may show distinctly different reactivities towards H_2O_2 so that equation (5) is applicable where M and D represent monomeric and dimeric

$$v_0 = k_M[M][H_2O_2] + k_D[D][H_2O_2] \quad (5)$$

forms of the ferrihaems respectively, which are in equilibrium [equation (6)].[9-11,13-15] Writing $k_1 = v_0/[H_2O_2]$ and combining equations (5) and (6) yields (7).

$$2M \overset{K}{\rightleftharpoons} D + H^+ \quad (6)$$

$$k_1/[M] = k_M + k_D(K/[H^+])[M] \quad (7)$$

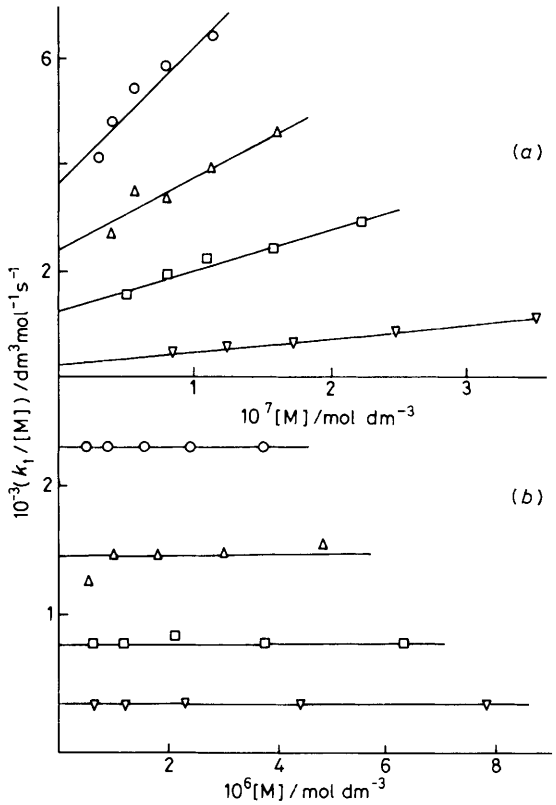


Figure 1. Kinetic data for protoferrihaem (*a*) and coproferrihaem (*b*) plotted according to equation (7) at pH 7.0 (▽), 7.4 (□), 7.75 (△), and 8.05 (○)

Figure 1 illustrates the two types of behaviour observed when the data were treated according to equation (7). Values of [M] at each value of [T] were calculated at the relevant pH from the known values of K (Table). For pfh (Figure 1) and also for mfh, plots of the data according to equation (7) were

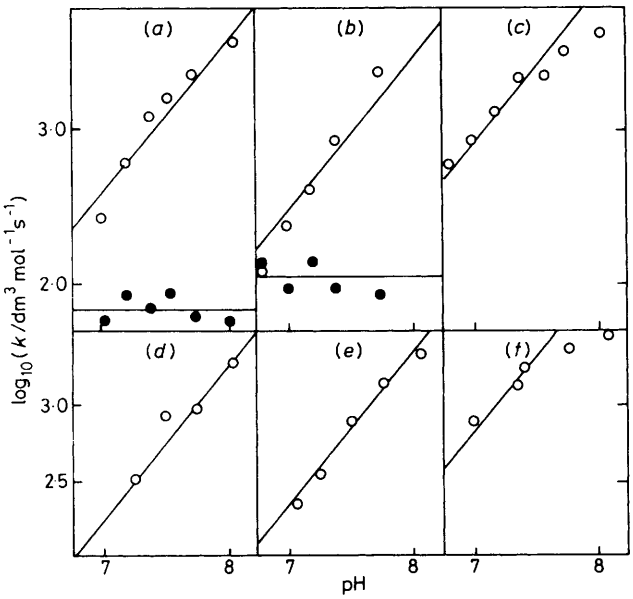


Figure 2. Influence of pH on k_M (○) and k_D (●) for pfh (*a*), mfh (*b*), dfh (*c*), hfh (*d*), cfh (*e*) and dds (*f*)

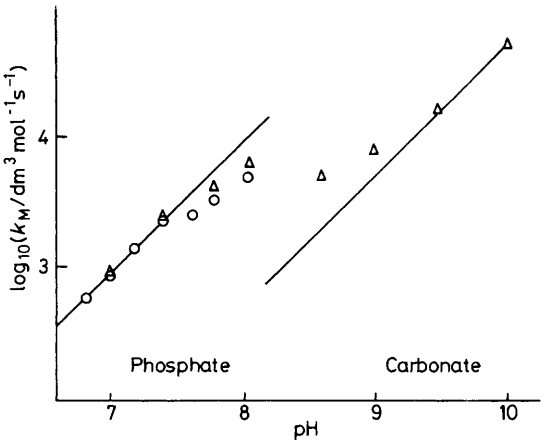


Figure 3. Values of k_M for deuteroferrihaem obtained in phosphate and carbonate buffers using hydroquinone (○) and ascorbic acid (△) as reductants

linear with a positive slope and positive intercept on the ordinate indicating $k_M > 0 < k_D$. For cfh (Figure 1), dfh, hfh, and dds the plots according to equation (7) were also linear but the slope was zero within experimental error, *i.e.* for these systems $k_M > 0$ but $k_D = 0$ (or at least $k_M \gg k_D$). Calculated standard deviations for the values of k_M and k_D were in the range ±10—15%.

The influence of pH on the values of k_M and k_D for all systems is shown in Figure 2. For four systems (pfh, mfh, hfh, and cfh) an inverse dependence of k_M on $[H^+]$ (solid lines of unit positive slope in Figure 2) satisfactorily describes the data. This is also the case for dfh and dds at pH < 7.5 but negative deviations from unit slope appear at higher pH. Similar effects have been observed with dfh in studies both of the overall catalatic reaction [2] and of intermediate formation by stopped-flow spectrophotometry [16] and have been associated with specific phosphate buffer effects. Data over an extended pH range have been obtained for dfh by using ascorbic acid as reductant. The results (Figure 3) show that the values of k_M in phosphate buffers are similar with both reductants, as expected. In carbonate buffers the values of k_M also show an inverse dependence on $[H^+]$ at high pH but are not collinear with the results in phosphate buffers. This behaviour is very similar to that observed in the catalytic reaction.[2] At pH < 7.5 in phosphate buffers where, for all systems, $k_M \propto [H^+]^{-1}$, the values of k_M for different ferrihaems although similar are not identical. The reactivity sequence is dfh > dds > pfh ≈ mfh > cfh ≈ hfh with a five-fold increase in k_M from hfh to dfh.

The reactions of the dimers of pfh and mfh show a similar behaviour in that, for both systems, k_D is independent of pH. However, whereas for pfh $k_M > k_D$ in the range of pH studied, the reactivity of dimeric mfh (which is almost twice that of dimeric pfh) is comparable to that of monomeric mfh at pH < 7.

Studies of the catalytic activity of ferrihaems in the decomposition of H_2O_2 have shown that the total activity may be represented as a sum of contributions associated with monomeric ferrihaems (a_M) and dimeric ferrihaem species (a_D). The relationship between a_M and k_M is, in general, complex, since a_M is a composite quantity involving the rate constants both for intermediate formation and intermediate reduction. For example, in the classical ' peroxidatic ' mechanism of catalase action [equations (1) and (2)] the overall catalytic rate constants $k_0 = k_1k_4/(k_1 + k_4)$ and the fraction of enzyme haem converted into oxidized intermediate in the steady state is $f_{ss} = k_1/(k_1 + k_4)$. Limiting cases for this mechanism arise if either (*a*) $k_1 \gg k_4$ when $f_{ss} \longrightarrow 1$ or (*b*) $k_1 \ll k_4$ when $f_{ss} \longrightarrow 0$. For the catalase enzymes themselves $k_1 \approx k_4$ so that appreciable formation of Compound I occurs. Although the detailed mechanism of ferrihaem catalysis (which will be discussed elsewhere [17]) is more complex, the behaviour of dfh appears in this respect similar to that of the enzymes in that appreciable, but sub-stoicheiometric, formation of intermediate is observed [6] in the reaction with H_2O_2. In contrast, in the reaction of dds with H_2O_2 no formation of intermediate is spectroscopically observable [15] although k_M is comparable to that with dfh, and appreciable formation of intermediate is observed in the reaction of dds with peroxyacids. Reaction of dfh with peroxyacids occurs essentially stoicheiometrically.[6] Although the data are, as yet, incomplete, it appears that reduction of the oxidized ferrihaem intermediate by H_2O_2 is also appreciably influenced by the porphyrin structure. Values of a_M show an inverse dependence on $[H^+]$, similar to k_M, but the influence of porphyrin structure on a_M does not follow the same sequence [for a_M: cfh > pfh > dfh ≈ mfh, with a_M(cfh) ≈ $4a_M$(mfh)]. Interestingly, although attempts to study the overall catalytic action of hfh were frustrated by an unexplained anomalous behaviour,[3] no complications appeared in the determination of k_M for hfh.

From the present data, formation of an oxidized intermediate by reaction of H_2O_2 with dimeric ferrihaems (k_D) was only observed with pfh and mfh, although significant values of a_D have been reported [3] for dfh and cfh in addition to pfh and mfh. Whereas k_D is independent of pH (Figure 2), a_D shows a complex pH dependence [3] (a_D approximately proportional to $[H^+]^{-0.5}$). Studies of the spectrum [6] and kinetics of reduction [18] of the oxidized derivative of dfh indicate that the monomeric intermediate reacts with unoxidized dfh to form a mixed-oxidation-state dimer which may show very different oxidizing properties, *e.g.* phenol reacts only with the monomeric intermediate. Thus contributions to the overall catalytic rate constant from dimeric species may arise in both the catalyst oxidation (k_D) and catalyst reduction phases of the cycle.

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