Reactions of Peroxy Acids with Deuteroferrihaem†

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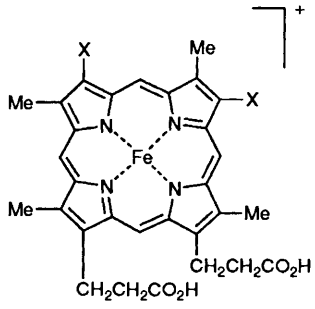
Deuteroferrihaem reacts with *m*-chloroperoxybenzoic acid and monoperoxosulfate in a 2:1 stoichiometry whilst it reacts with peroxyacetic acid in a 4:1 stoichiometry. The UV/VIS spectra of the haem intermediates appear to be the same in all cases despite the different stoichiometries. The reaction of deuteroferrihaem and monoperoxosulfate is slow enough to obtain repetitive UV/VIS spectrophotometric scans during the formation of the intermediate as well as the regeneration of the ferrihaem. It is notable that, even with a significant initial excess of peroxy acid, ferrihaem regeneration proceeds immediately after intermediate formation. Thus there is no peroxy acid turnover phase. Instead the build up of a primary degradation product characterised by a broad absorbance band in the visible is observed at higher peroxy acid concentrations. A kinetic scheme for the formation of the intermediate and regeneration of ferrihaem is described that explains the amount of intermediate formation and the rate and extent of haem regeneration as a function of the nature and concentration of the peroxy acid.

Derivatives of the naturally occurring iron(III) complex of protoporphyrin IX, protoferrihaem, **1a**, exhibit catalytic properties in aqueous solution that, depending on pH, are similar to the haemoproteins catalase and peroxidase.[1] Studies of the protein-free derivatives of natural ferrihaems, such as deutero-, meso- and copro-ferrihaem (**1b**,**1c** and **1d** respectively, represented as $[FeP]^+$, where P is any porphyrinato dianion and charges on ionised propionic acid ring substituents are ignored) are complicated by pH-dependent μ-oxo dimer, *i.e.* PFeOFeP, formation reactions whose equilibrium and kinetics are, however, well characterised.[2,3] The rate-limiting step for the reaction of deuteroferrihaem,[4] and other ferrihaems[5,6] with hydrogen peroxide, peroxy acids, and other oxygen-transfer agents is dependent on the concentration of ferrihaem monomer and that of the peroxide.[4-6] The reaction product oxidises substrates such as phenols, alcohols, aromatic amines and halides, with concomitant regeneration of ferrihaem.[1,5,7,8] In the absence of added substrate a relatively slow spontaneous partial regeneration of the ferrihaem occurs.[5,7,9] Because of these regeneration reactions the observed product of the reaction of ferrihaem and peroxide is referred to as an 'intermediate'. Stopped-flow spectrophotometric titrations and repetitive-run stopped-flow spectral scanning showed that the stoichiometry of the reaction between deuteroferrihaem and peroxybenzoic acids was 2:1 yielding an intermediate with a λ_{max} about 320 nm.[10] One of the possibilities suggested for the nature of the predominant intermediate species was $[PFeOFeP]^{2+}$, following the earlier report of the sequential electrochemical oxidation of the iron(III) centres of synthetic porphyrin μ-oxo dimer complexes in CH_2Cl_2 to form $[PFeOFeP]^+$ and $[PFeOFeP]^{2+}$ species.[11]

In the present paper we describe an integrated study of intermediate formation and ferrihaem regeneration for the reaction of deuteroferrihaem and the peroxy acids *m*-chloroperoxybenzoic acid, monoperoxosulfate, $HO_2SO_3^-$, and peroxyacetic acid. With peroxyacetic acid we see a 4:1 haem to peroxy acid stoichiometry which corresponds to a $[PFeOFeP]^+$ intermediate, yet the intermediate has a virtually identical spectrum to that formed in the 2:1 stoichiometric reactions with the other peroxy acids. We interpret these results in terms of the reaction of a second peroxide molecule as has been reported for a synthetic porphyrin[12] and discussed for horseradish peroxidase compound 0.[13]



X
1a $CHCH_2$
b H
c CH_2CH_3
d $CH_2CH_2CO_2H$

Experimental

Materials.—Deuteroferrihaem was prepared using a modification of the resorcinol-melt method of Falk.[14] A well ground mixture of protoferrihaem 2.0 g, purchased from Fluka A G, and resorcinol (6.0 g) was heated under air reflux in an oil-bath at 150–155 °C for 45–55 min and allowed to cool. In our modification the crude product was washed with diethyl ether in a Soxhlet extractor for at least an hour to yield a dull brown powder. This was recrystallised using a procedure adapted from Caughey *et al.*[15] Some of the crude product, 0.5 g, was dissolved in pyridine (2.50 cm^3). To this was added chloroform (3.75 cm^3) and the mixture mechanically shaken for 15 min. The solution was filtered using a fluted filter paper and the paper washed with chloroform (1.0 cm^3). The filtrate was then poured into a previously made up, boiling, solution of glacial acetic acid

† Deutero-, proto-, meso- and copro-porphyrin = 3,7,12,17-tetramethyl-, 3,7,12,17-tetramethyl-8,13-divinyl- and 7,12-diethyl-3,8,13,17-tetramethyl-porphyrin-2,18-dipropanoic acid and 2,7,12,18-tetramethyl-3,8,13,17-tetrapropanoic acid.

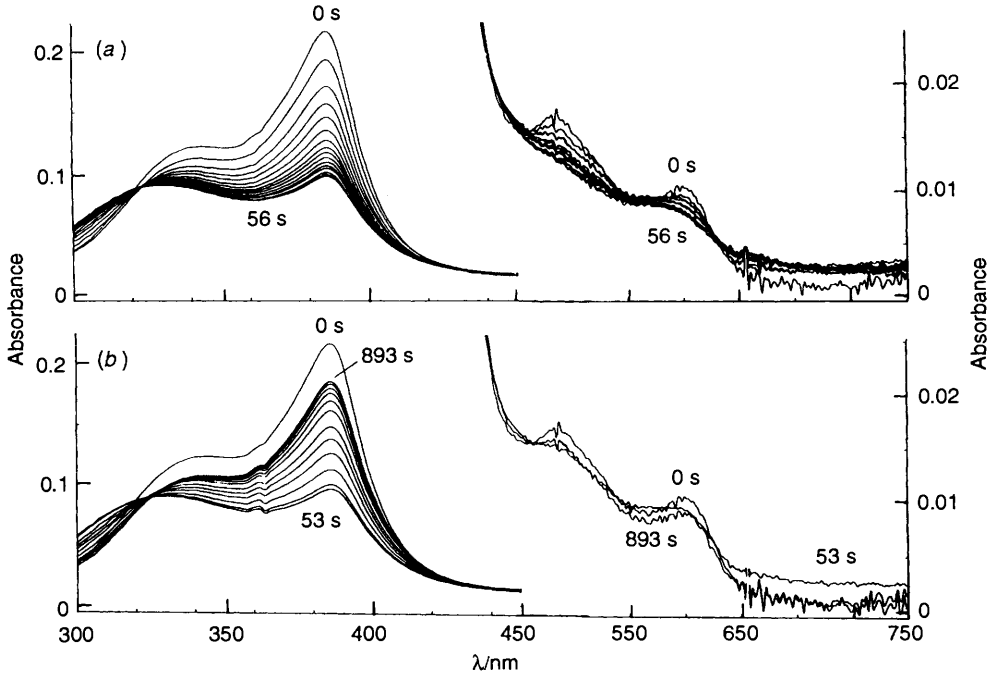


Fig. 1 Spectrum of deuteroferrihaem (2.75×10^{-6} mol dm^{-3}) and sequential spectra during its reaction with monoperoxosulfate (1×10^{-6} mol dm^{-3}) over (*a*) 3–56 and (*b*) 53–893 or 53 and 893 s. Phosphate buffer, 0.01 mol dm^{-3}, pH 7.4, *I* 0.1 mol dm^{-3} with $NaNO_3$ at 25 °C

(40.0 cm^3), saturated sodium chloride solution (0.75 cm^3) and concentrated hydrochloric acid (0.50 cm^3) that had been filtered before use. The resultant solution was allowed to stand for 12 h, whereupon black crystals appeared on the bottom of the flask. These were filtered off from the solution using a Buchner funnel under vacuum and were washed with 50% aqueous glacial acetic acid (7.5 cm^3), distilled water (12.5 cm^3), ethanol (3.0 cm^3) and diethyl ether (3.0 cm^3). The crystals were left to dry in an oven at a temperature not greater than 50 °C. After drying the deuteroferrihaem appeared as shiny, black, finely divided crystals with a recrystallisation yield from the original 0.5 g of crude product of 39%. A second recrystallisation was performed on the crystals obtained from the first recrystallisation with a yield of 60%. The deuteroferrihaem was pure by Macherey and Nagel (MN) polyamide chromatography.[16]

Stock solutions of deuteroferrihaem, about 1×10^{-4} mol dm^{-3} were prepared by dissolving a weighed amount of the solid in a minimum volume of NaOH (0.1 mol dm^{-3}) and diluting volumetrically with NaH_2PO_4–Na_2HPO_4 (pH 7.4, 0.01 mol dm^{-3}) buffer, ionic strength 0.1 mol dm^{-3} with $NaNO_3$. Significantly higher concentrations of phosphate caused precipitation of haem in the stock solutions.

Potassium monoperoxosulfate triple salt, $2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$ and 40 wt% peroxyacetic acid, Proxitane 4002, were donated by Interox Chemicals. Hydrogen peroxide was removed from the Proxitane simply by raising the pH of a diluted solution to 10.5 and leaving for 5 min before adjusting the pH to the required value.[17] The virtual absence of hydrogen peroxide in the resulting solution over the period of its use was confirmed cerimetrically. The *m*-chloroperoxybenzoic acid (80%, with the parent acid as the only significant impurity) was purchased from Sigma. Peroxy acid stock solutions were made up daily and determined iodometrically immediately before dilution to the required concentration.

Methods.—All measurements were made at 25 °C in NaH_2PO_4–Na_2HPO_4 (pH 7.4, 0.01 mol dm^{-3}) buffer, ionic strength adjusted to 0.1 mol dm^{-3} with $NaNO_3$. Absorbance scans were obtained using a Hewlett-Packard HP8451A diode-array spectrophotometer fitted with a 295 nm cut-off filter which effectively eliminated photodegradation of the haem during repetitive measurements. Single wavelength measurements were made with a Hi-Tech SF4 stopped-flow spectrophotometer connected to a personal computer fitted with a PCL-812 12 bit interface card. Programs were written to allow analysis of mono- and multi-exponential changes in absorbance with time using non-linear regression. Additionally, the absorbance *versus* time data were fitted to a cubic spline function whose derivative gave the rate.

Results

The reaction of deuteroferrihaem with monoperoxosulfate at pH 7.4 is slow enough to scan repetitively on the diode-array spectrophotometer. Fig. 1(*a*) shows the spectral changes in the first minute after mixing haem and peroxy acid to give concentrations of about 3×10^{-6} and 1×10^{-6} mol dm^{-3}, respectively. There is an initial, rapid, but small, drop in the absorbance in the 300–350 nm region. This is followed by a substantial drop in the 384, 490 and 600 nm bands with approximate isosbestic points at 325 and 630 nm. Fig. 1(*b*) shows the regeneration of haem over the next 15 min. It is notable that the same isosbestic points are present during the regeneration. The final spectrum represents about 90% regeneration of the haem and there is no indication of degradation products absorbing in the 300–800 nm region. Fig. 2 shows the spectral changes at similar times after mixing haem and peroxy acid to give concentrations of about 3×10^{-6} and 6×10^{-6} mol dm^{-3}, respectively. The absorbance in the 320–420 nm region drops rapidly to give a spectrum very similar to that observed previously for the reaction of deuteroferrihaem and *m*-chloroperoxybenzoic acid using repetitive-run stopped-flow spectral scanning.[10] At the same time the bands at 490 and 600 nm are rapidly lost. There are no isosbestic points during this phase of the reaction. Fig. 2(*a*) also shows that subsequent to this the absorbance continues to drop in the 320 nm region, drops less markedly around 384 nm and a broad absorbance develops above about 450 nm. Fig. 2(*b*) shows the subsequent haem regeneration. The isosbestic point at 325 nm is once again

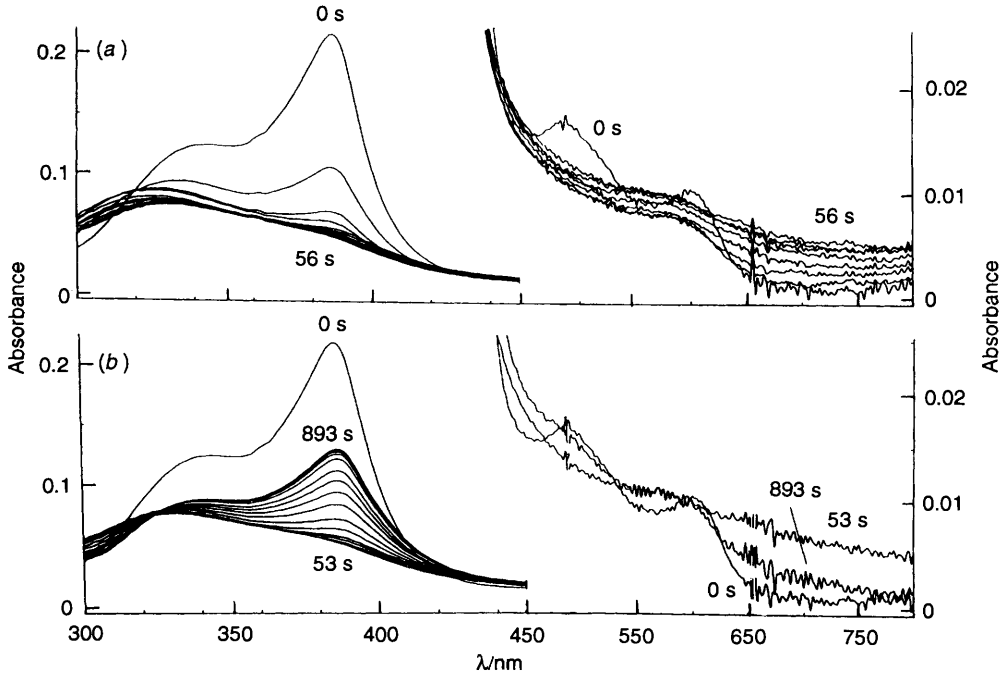


Fig. 2 Spectrum of deuteroferrihaem (2.75 × 10^{-6} mol dm^{-3}) and sequential spectra during its reaction with monoperoxosulfate (6 × 10^{-6} mol dm^{-3}) over (*a*) 3–56 and (*b*) 53–893 or 53 and 893 s. Conditions as in Fig. 1

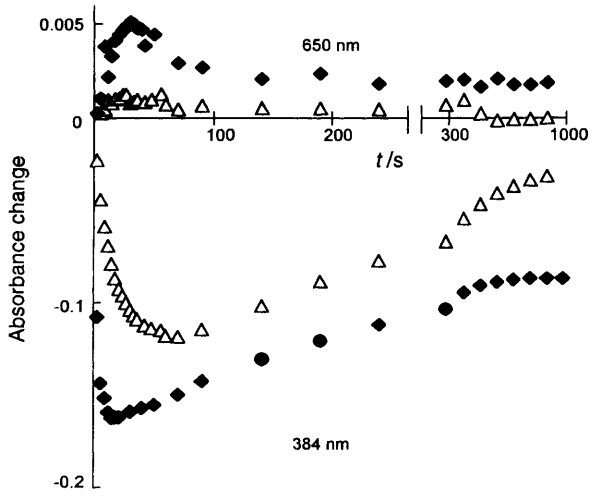


Fig. 3 Time course of the absorbance changes at 650 and 384 nm during the reaction of deuteroferrihaem (2.75 × 10^{-6} mol dm^{-3}) and monoperoxosulfate (1 × 10^{-6} △ and 6 × 10^{-6} mol dm^{-3} ◆). The data are taken from Figs. 1 and 2

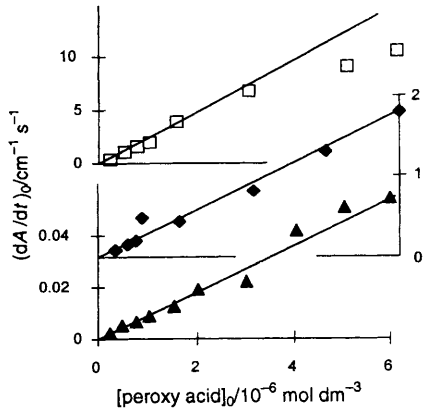


Fig. 4 Effect of initial peroxy acid concentration on the initial rate of absorbance loss at 384 nm during reaction with deuteroferrihaem: □ *m*-chloroperoxybenzoic acid, ◆ peroxyacetic acid, ▲ monoperoxosulfate. Conditions as Fig. 1

apparent and the broad absorbance in the visible gradually drops. The final spectrum represents about 70% regeneration of deuteroferrihaem with a residual absorbance due to the broad band in the visible representing a coloured degradation product. The time-courses of the absorbance changes at 384 and 650 nm taken from Figs. 1 and 2 are shown in Fig. 3. The concerted nature of the absorbance changes at the two respective wavelengths at the lower peroxy acid concentration is emphasised, whereas at the higher peroxy acid concentration the absorbance at 650 nm continues to rise after that at 384 nm has reached a minimum. An interesting feature of the absorbance changes in the 384 nm region at the higher peroxy acid concentration shown in Fig. 3 is the absence of a broad minimum in the absorbance–time course corresponding to a peroxy acid turnover phase that might be expected with excess peroxy acid. The absence of such peroxy acid turnover phases is even more striking in the case of the stopped-flow traces (not shown) at 384 nm with excess peroxyacetic and *m*-chloroperoxybenzoic acids where intermediate formation is so much faster than haem regeneration.

Intermediate formation from the reaction of deuteroferrihaem and peroxyacetic or *m*-chloroperoxybenzoic acids was too rapid to be followed on the diode-array spectrophotometer. However, the subsequent regeneration of the haem was followed on the diode array and all of the qualitative features of the monoperoxosulfate results were apparent (data not shown). Figs. 4–6 represent a summary of stopped-flow runs at 384 nm for all three peroxy acids. Fig. 4 shows that the initial rate of reaction of haem and peroxy acid is directly proportional to the concentration of the peroxy acid, with, perhaps, some evidence for the onset of rate saturation in the case of *m*-chloroperoxybenzoic acid. The second-order rate constant for *m*-chloroperoxybenzoic acid, (2.3 ± 0.1) × 10^6 dm^3 mol^{-1} s^{-1} cm^{-1} is

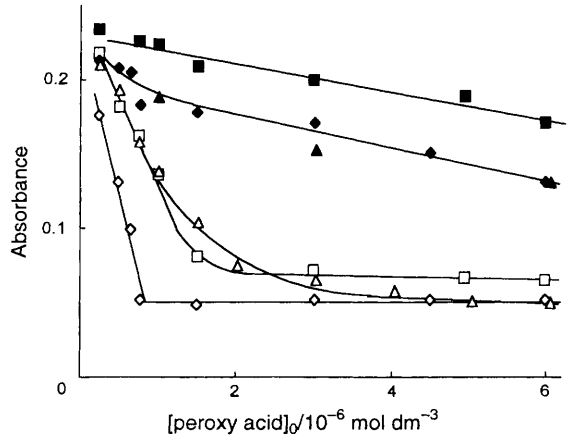


Fig. 5 Effect of initial peroxy acid concentration on the absorbance minimum at 384 nm, corresponding to intermediate formation (□, ◇, △) and on the final absorbance at 384 nm, corresponding to the extent of haem regeneration (■, ◆, ▲): □, ■ *m*-chloroperoxybenzoic acid; ◇, ◆ peroxyacetic acid; △, ▲ monoperoxosulfate. Conditions as Fig. 1. The lines are for a guide only

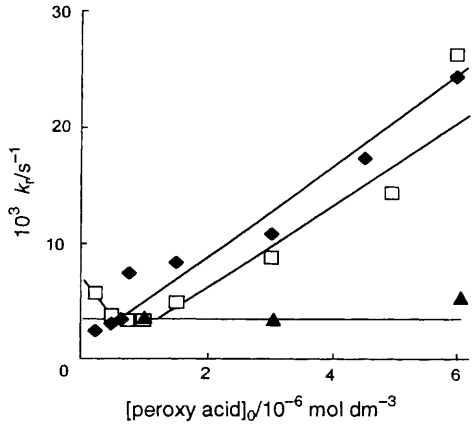


Fig. 6 Effect of initial peroxy acid concentration on the rate constant for regeneration of haem: □ *m*-chloroperoxybenzoic acid, ◆ peroxyacetic acid, ▲ monoperoxosulfate. Conditions as Fig. 1. The lines are for a guide only

consistent with previous measurements[18] and considerably larger than that of peroxyacetic acid, (2.98 ± 0.05) × 10^5 dm^3 mol^{-1} s^{-1} cm^{-1}, which, in turn, is considerably faster than monoperoxosulfate, (9.5 ± 0.4) × 10^3 dm^3 mol^{-1} s^{-1} cm^{-1}.

Fig. 5 shows the minimum absorbance obtained during the run, corresponding to the formation of the intermediate, and also the maximum absorbance obtained after haem regeneration. The former measurements represent the spectrophotometric titrations described previously whilst the latter can be used as an indication of the amount of haem degradation. Extrapolation of the haem degradation curves in Fig. 5 suggests that complete degradation of the haem would occur at about an 8-fold or 9-fold excess of peroxy acid. The spectrophotometric titration for *m*-chloroperoxybenzoic acid yields the approximate 2:1 haem to peroxy acid stoichiometry observed previously.[10] A similar result is obtained for the monoperoxosulfate titration. Here the more gradual break is consistent with the slower intermediate formation reaction. The most striking result in Fig. 5 is that compared with the other peroxy acids, peroxyacetic acid shows a higher stoichiometry of more than 3:1 haem to peroxy acid. Spectral scans during haem regeneration, started as soon after mixing as possible, show that the resultant spectral changes with peroxyacetic acid are identical to those with *m*-chloroperoxybenzoic acid, including the 325 nm isosbestic, which suggests that the same absorbing species are involved in the reactions of both peroxy acids. The only difference is the smaller amount of peroxyacetic acid required to induce the changes, consistent with the different stoichiometry we have already described. Fig. 5 also shows that the amount of haem degradation with peroxyacetic acid is similar to that with monoperoxosulfate, which is slightly more than with *m*-chloroperoxybenzoic acid. Thus the stoichiometry change with peroxyacetic acid is not a simple direct consequence of the extent of haem degradation. Neither does the stoichiometry change with peroxyacetic acid correlate with the different haem regeneration rates, which Fig. 6 shows are similar for peroxyacetic and *m*-chloroperoxybenzoic acids and rather less for monoperoxosulfate.

The first-order rate constants for haem regeneration plotted in Fig. 6 were obtained by fitting the increase in absorbance at 384 nm to a single exponential process. Fig. 6 shows that the rate constant for regeneration of haem from the intermediate formed with peroxyacetic acid is proportional to the initial concentration of peroxy acid, whereas with monoperoxosulfate it is virtually independent. The regeneration behaviour with *m*-chloroperoxybenzoic acid is more complex, at low initial peroxy acid concentrations the rate constant is independent of, or even inversely proportional to, the initial concentration of peroxy acid whilst at higher peroxy acid concentrations the rate constant for regeneration increases with increasing peroxy acid concentration. The kinetics of deuteroferrihaem regeneration from the intermediate formed with *m*-chloroperoxybenzoic acid have been reported.[9] Here reactions were carried out at higher pH and the regeneration was about 10 times faster and biphasic.

As part of our investigation of the different stoichiometry seen with peroxyacetic acid we carried out experiments utilising an appropriately-calibrated Rank oxygen electrode and deuteroferrihaem at 3 × 10^{-5} mol dm^{-3} in partially deoxygenated buffer solutions. These showed that oxygen is not evolved during the course of the reaction of haem and *m*-chloroperoxybenzoic acid. On the other hand, neither is oxygen consumed during the course of the reaction of peroxyacetic acid and haem. In all, no evolution or consumption of oxygen was observed in any of these experiments.

Discussion

The apparent 2:1 deuteroferrihaem:substituted-peroxybenzoic acid stoichiometry we originally observed in aqueous solution[10] has prompted a number of similar studies of the reactions of peroxides and other oxygen-atom donors with derivatives of the naturally occurring protoferrihaem.[4–7,9,10] Of related interest are mechanistic studies of the haem c octapeptide known as microperoxidase-8,[19] and spectroscopic studies of high oxidation-state intermediates of horseradish peroxidase and the *N*-acetyl derivatives of microperoxidase.[13,20] Also of interest are the reactions of soluble synthetic tetraarylferriporphyrins with peroxides including monoperoxosulfate and *m*-chloroperoxybenzoic acid in aqueous solution.[21] The intriguing point of comparison between the natural haem derivatives and the group consisting of horseradish peroxidase itself, microperoxidase and the water-soluble synthetic haems is as follows. Whereas the latter group, in the course of their reactions with peroxides, all form intermediates that exhibit the spectroscopic properties of a species that can reasonably be assigned as a peroxidase compound II analogue, [FeP]$^{2+}$, the UV/VIS spectra of the intermediates formed from natural haem derivatives and peroxides are quite different from those of the other group. Moreover, this is notwithstanding that the stoichiometry of the deuteroferrihaem peroxybenzoic acid reaction is 2:1, which is the stoichiometry expected for a compound II analogue.

The reaction sequence in Scheme 1 models the major features of the kinetic data. Axial ligands, except for the postulated bridging oxo groups, are omitted. The deuteroferrihaem

Table 1 Rate constants for reactions in Scheme 1

	$k_1/10^6$ dm^3 mol^{-1} s^{-1}	$(k_2/k_3)/$ 10^6 dm^3 mol^{-1}	$k_5/10^2$ dm^3 mol^{-1} s^{-1}	$k_6/10^3$ dm^3 mol^{-1} s^{-1}	$k_7/10^3$ dm^3 mol^{-1} s^{-1}	$k_9/10^4$ dm^3 mol^{-1} s^{-1}
m-$ClC_6H_4CO_3H$	8.87	20	2	30	3	2
$MeCO_3H$	1.15	0.001	2	30	3	2
$HO_2SO_3^-$	0.0386	2	2	5	3	2

monomer–dimer equilibrium is pH dependent and the value of the equilibrium constant, K_d, 8.54×10^5 dm^3 mol^{-1} is calculated at pH 7.4 from ref. 3. The limiting rate constant, k_1, is expressed in terms of the concentration of monomeric haem and protonated peroxy acid, XO_2H. It is calculated from the initial rate data shown in Fig. 4 according to equation (11).

$$[FeP]^+ + [FeP]^+ \overset{K_d}{\rightleftharpoons} [PFeOFeP]$$

$$[FeP]^+ + XO_2H \xrightarrow{k_1,\ \text{rate limiting}} \mathbf{I}' \quad (1)$$

$$\mathbf{I}' + XO_2H \xrightarrow{k_2,\ \text{fast}} \mathbf{I} + XO_2X \quad (2)$$

$$\mathbf{I}' \xrightarrow{k_3,\ \text{fast}} \mathbf{I} \quad (3)$$

$$\mathbf{I} + 3[FeP]^+ \xrightarrow{\text{fast}} 2[PFeOFeP]^+ \quad (4)$$

$$[PFeOFeP]^+ + [FeP]^+ \xrightarrow{k_5,\ \text{rate limiting}} 2[FeP]^+ + \mathbf{A} \quad (5)$$

$$[PFeOFeP]^+ + XO_2H \xrightarrow{k_6,\ \text{rate limiting}} [FeP]^+ + \mathbf{A} \quad (6)$$

$$[PFeOFeP]^+ + \mathbf{A} \xrightarrow{k_7,\ \text{rate limiting}} 2[FeP]^+ + \mathbf{B} \quad (7)$$

$$2n[PFeOFeP]^+ + \mathbf{B} \xrightarrow{\text{fast}} 4n[FeP]^+ + \mathbf{C} \quad (8)$$

$$XO_2H + \mathbf{A} \xrightarrow{k_9,\ \text{rate limiting}} \mathbf{B} \quad (9)$$

$$nXO_2H + \mathbf{B} \xrightarrow{\text{fast}} \mathbf{C} \quad (10)$$

Scheme 1

$$k_1 = \frac{(dA/dt)_0}{4[HO_2X]_0 \Delta A_{max} \alpha_0} \quad (11)$$

Here α_0 is the degree of dissociation of the haem calculated from K_d at the initial haem concentration and ΔA_{max} is the maximum absorbance change estimated from the absorbance minima shown in Fig. 5 and corresponds to complete conversion of ferrihaem to intermediate, which is represented as $[PFeOFeP]^+$ in Scheme 1. The stoichiometric number, 4, in equation (11) reflects the reaction of three further $[FeP]^+$ units in the non-rate-limiting step (4) which obviously represents a sequential process. Values of k_1 for the three peroxy acids are shown in Table 1. The differing stoichiometry with different peroxy acids is explained by steps (2) and (3). The intermediate, **I**′, formed in the initial, essentially irreversible, rate-limiting step (1) is a haem–peroxy acid adduct in which the integrity of the peroxide bond is open to conjecture.[13] This intermediate may react with a second peroxy acid in the non-rate-limiting step (2) to form the intermediate, **I**, which is at the same overall oxidation level as **I**′, and involves the release, therefore, of a disubstituted peroxide, XO_2X. This process has been observed in synthetic ferrihaems.[12] Alternatively **I**′ may convert directly to **I** in a non-rate-limiting step such as step (3).[13] The implications of such a change in pathway with peroxide concentration have been discussed with respect to compound 0 formation with horseradish peroxidase.[13] The constants k_2, k_3 and k_4 are non rate-limiting and so do not affect the overall rate of formation of $[PFeOFeP]^+$, thus their values are not required for the model. The relative magnitudes of $k_2[XO_2H]$ and k_3 affect the overall stoichiometry of the reaction with respect to XO_2H, however, and so the quotient k_2/k_3 is required for modelling. The high-oxidation-state intermediates produced in steps (2) and (3) are identically designated as **I**. This is sufficient for modelling the major features of the kinetic data but, obviously, may not actually be the case. The initial rapid but small drop in the absorbance in the 300–350 nm region shown in Fig. 1(*a*) is indicative of the onset of a steady state in one or more intermediates such as **I** or **I**′. In our model we have made the steady state approximation in **I** and **I**′. This ensures that the system of differential equations associated with this reaction sequence never becomes stiff, with the associated practical difficulties in the numerical solution.[22] Moreover, this proposed sequence does not include any steps that involve species that are not experimentally observable, unless they are required to provide the experimentally measured stoichiometry.

The formation of primary degradation products (**A**) characterised by a broad visible absorbance, is represented by steps (5) and (6). These steps are likely to involve a pre-equilibrium that is the reverse of the processes involved in step (4) and a steady state or states in the higher oxidation-state haem species, **I** or **I**′. Step (4) represents a sequence of steps and it is not implied that its reverse involves a bimolecular reaction of the observed intermediate species, $[PFeOFeP]^+$. Steps (5) and (6) explain why there is not observed a build up of higher-oxidation-state intermediates, **I** and **I**′. These either oxidise the haem or are themselves oxidised by peroxy acid to yield primary degradation products which, in their turn, are oxidised to secondary oxidation products (**B**) in steps (7) and (9). Step (7) resembles step (5) in that a pre-equilibrium involving the formation of a higher-oxidation-state intermediate is likely. The rapid steps (8) and (10) are included to allow for the stoichiometric amount of peroxy acid completely to bleach the haem to colourless degradation products (**C**).

The differential equations with the stoichiometric number, *n*, assumed to be 3, were solved numerically using the 4th order Runge Kutta method.[22] The absorbance at 384 nm was calculated assuming that the absorption coefficient of the observed intermediate, on a per iron basis, was 0.25 times that of the deuteroferrihaem and that the absorption coefficient of the primary degradation product was 0.2 times that of deuteroferrihaem. For each peroxy acid the value of k_1 calculated according to equation (11) from the data in Fig. 4 was substituted into the differential equation together with values

of k_2/k_3, k_5, k_6, k_7 and k_9 chosen by trial and error until reasonable agreement with the kinetic results in Figs. 5 and 6 was obtained. These values are included in Table 1. The only feature of the kinetic results which Scheme 1 failed to model was the inverse dependence of haem regeneration on peroxy acid concentration at low concentrations of *m*-chloroperoxybenzoic acid, shown in Fig. 6. Such behaviour suggests that at low peroxy acid concentration there is a greater amount of a more reactive, in terms of haem degradation, high-oxidation-state intermediate than at higher peroxy acid concentration; removal of some of the steady-state criteria would account for this, for example, if **I'** was more strongly oxidising than **I**, or if the product of step (3) was different from and more strongly oxidising than the product of step (2). Although Scheme 1 provides a reasonably good model, and hence explanation, of the processes occurring on reaction of deuteroferrihaem and peroxy acids, it is unlikely to be entirely correct in detail. There are a number of alternative steps that would yield similar model results and hence we have made no attempt to optimise the fitting procedure to obtain best estimates of the rate-determining rate constants.

The fact that O_2 is not generated during the reaction of *m*-chloroperoxybenzoic acid and deuteroferrihaem led us to the conclusion that step (2) involves the production of a dibenzoyl peroxide, or peroxodisulfate in the case of monoperoxosulfate, as observed for a synthetic ferrihaem.[12] The fact that oxygen is not consumed in the reaction of deuteroferrihaem and peroxyacetic acid is evidence against homolytic peroxide-bond cleavage[23] to form an acetyl radical with the subsequent formation of carbon dioxide and a reducing methyl radical that would directly or indirectly consume oxygen.[24]

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