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Reaction of deuteroferrihaem and *m*-chloroperoxybenzoic acid in surfactant micelles†

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The effects of non-ionic and anionic surfactant micelles on the UV/VIS spectrum of deuteroferrihaem and the kinetics of its reaction with *m*-chloroperoxybenzoic acid in pH 7.4 phosphate buffer have been studied. The results were analysed in terms of the micellar association constants of the haem and the transition state. The spectra and association constants are discussed with regard to the effect of surfactant on the ionization and dimerization equilibria of the haem. The visible region of the deuteroferrihaem spectrum changes much more in the presence of Triton X-100 than sodium dodecyl sulfate (sds). The effect of sds concentration on the observed second-order rate constant for the reaction was corrected for the micellar association of the peroxy acid and yielded micellar association constants for the transition state and the haem, K_{mic}^{TS} 150 ± 20 dm^3 mol^{-1} and $K_{mic}^{haem,\ pH\ 7.4}$ 720 ± 80 dm^3 mol^{-1}, respectively. The latter value is in good agreement with the value of the micellar association constant 870 ± 100 dm^3 mol^{-1} obtained from the effect of sds concentration on the absorbance in the Soret region of the UV/VIS spectrum of the haem. A similar treatment of the kinetics in Triton X-100 showed that the ratio of K_{mic}^{TS} to $K_{mic}^{haem,\ pH\ 7.4}$ increases from 0.06 to 0.14 : 1 with increasing surfactant concentration, compared to a constant ratio of 0.21 : 1 in sds. The effect of Triton X-100 concentration on the absorbance in the Soret region of the deuteroferrihaem spectrum is qualitatively similar to its effect on the reaction kinetics.

Derivatives of the naturally occurring iron(III) complex of protoporphyrin IX, protoferrihaem, exhibit catalytic properties in aqueous solution similar to those of the haemoproteins catalase and peroxidase.[1] Studies of these derivatives are complicated by pH-dependent dimer formation whose equilibria and kinetics are, however, well characterized.[2,3] The rate-limiting step for the reaction of deuteroferrihaem and other derivatives with hydrogen peroxide, peroxy acids, and other oxygen-transfer agents is dependent on the concentration of ferrihaem monomer and that of the peroxide.[4,5] We have recently described a detailed mechanism for the reaction of deuteroferrihaem and peroxy acids that is consistent with the kinetics, the spectrum of the long-lived intermediate species formed after the rate-limiting step, and the overall stoichiometry.[6] The present report extends these studies to the effect of the surfactants sodium dodecyl sulfate (sds) and α-[4-(1,1,3,3-tetramethylbutyl)phenyl]-ω-hydroxypoly(oxyethane-1,2-diyl) (Triton X-100) on the rate-limiting step of the reaction of deuteroferrihaem and *m*-chloroperoxybenzoic acid. The effects of the surfactants on the electronic spectrum of deuteroferrihaem are also described. The kinetics is interpreted in terms of transition-state stability[7] using a recently developed multiple micellar pseudo-phase model[8] based on the classical pseudo-phase kinetic model.[9]

Experimental

Materials and methods were as described previously.[6,8] All measurements were made at 25 °C in pH 7.4, 0.01 mol dm^{-3} NaH_2PO_4–Na_2HPO_4 buffer, ionic strength adjusted to 0.1 mol dm^{-3} with $NaNO_3$.

Results

Under these conditions of the experiments the critical micelle concentrations (c.m.c.s) of sds and Triton X-100 are 1.8 × 10^{-3} and 2.0 × 10^{-4} mol dm^{-3}, respectively. In the presence of 3.2 × 10^{-6} mol dm^{-3} deuteroferrihaem these values are only very slightly less at 1.3 × 10^{-3} and 1.9 × 10^{-4} mol dm^{-3}. Fig. 1 shows the effect of sds on the spectrum of *ca.* 3 × 10^{-6} mol dm^{-3} deuteroferrihaem. In buffer alone the λ_{max} values of the Soret band and two visible bands are 386, 491 and 604 nm, respectively. The corresponding values in 3 × 10^{-3} mol dm^{-3} sds are 390, 488 and 598 nm. In 2.6 × 10^{-2} mol dm^{-3} sds they are 392, 486 and 598 nm. The intensities of the bands increase with increasing sds concentration and the spectra at intermediate concentrations (not shown) do not exhibit any isosbestic points. Fig. 2 shows the effect of sds on the absorbance at 396 nm. There is little change below the c.m.c. and the subsequent increase in absorbance is treated semiquantitatively as follows. The micellar association of a species, R, with a surfactant, S, is often considered using equation (1) where the micellar

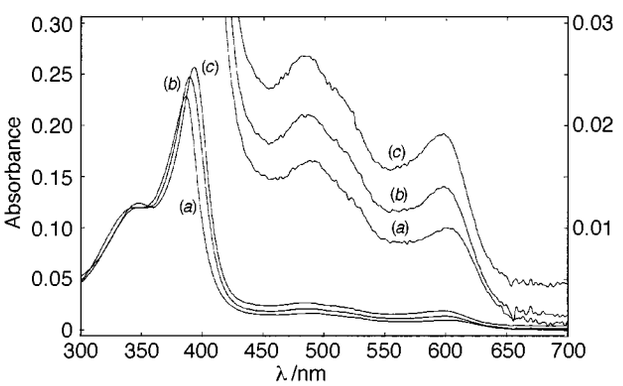

Fig. 1 Spectrum of 3.08 × 10^{-6} mol dm^{-3} deuteroferrihaem in (*a*) pH 7.4 buffer alone and in buffer containing 3 × 10^{-3} (*b*) and 2.6 × 10^{-2} mol dm^{-3} sds (*c*)

$$R + S \rightleftharpoons^{K_R} RS \quad (1)$$

association constant, K_R, as defined in equation (2), is expressed

† deuteroferrihaem = (3,7,12,17-tetramethylporphyrin-2,18-dipropionato)iron(III), protoferrihaem = (3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropionato)iron(III).

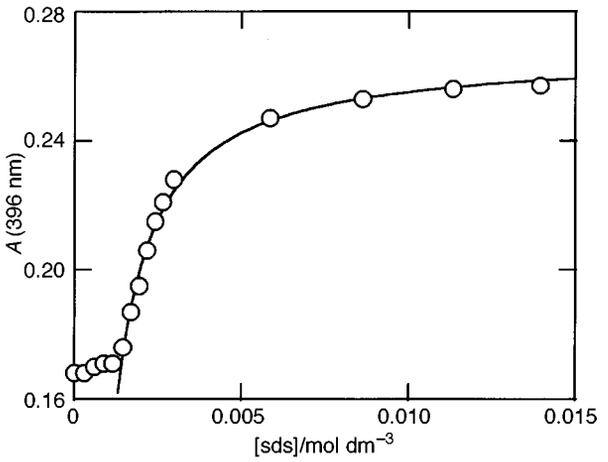

Fig. 2 Effect of sds concentration on the absorbance of deuteroferrihaem at 396 nm, conditions as in Fig. 1. The curve represents equation (3) with the best-fit parameters

$$K_R = [RS]/[R]([S] - \text{c.m.c.}) \quad (2)$$

in terms of the overall concentration of micellar surfactant. This leads to the expression (3) for absorbance, A, where a and

$$A = \frac{a + b([S] - \text{c.m.c.})}{1 + K_R([S] - \text{c.m.c.})} \quad (3)$$

b are functions of the absorption coefficients of R and RS. Fitting equation (3) to the data in Fig. 2 gives a value of the micellar association constant for deuteroferrihaem in sds of 870 ± 100 $dm^3\ mol^{-1}$ and the corresponding best-fit line is shown in the figure. This is obviously not a rigorous approach because the non-isosbestic spectra indicate the presence of more than two absorbing independent species. Nevertheless, the inverse of the micellar association constant obtained in this way provides an objective measure of the concentration of surfactant at which the absorbance change associated with the various equilibrium processes is half-complete.

Fig. 3 shows that the effect of Triton X-100 on the spectrum of deuteroferrihaem is different to that of sds. The intensity of the Soret band first decreases and then increases with increasing surfactant concentration and the visible region is markedly different. In 1×10^{-3} mol dm^{-3} Triton X-100 the λ_{max} values of the Soret band and major visible band are 388 and 568 nm, respectively. In 6×10^{-2} mol dm^{-3} Triton X-100 the corresponding values are 394 and 584 nm. Fig. 4 shows the effect of Triton X-100 on the absorbance at 384 nm. There is little change below the c.m.c. and the subsequent changes are treated semiquantitatively as two separable processes according to equation (3). This leads to micellar association constants of 8000 ± 2000 and 76 ± 14 $dm^3\ mol^{-1}$ and the corresponding best-fit lines shown in the figure. Again the approach is not rigorous because, first, isosbestic spectra are not obtained over either of the two concentration ranges (results not shown), secondly there is no evidence that the processes occurring in the different concentration ranges are actually separable, and thirdly it is difficult to conceive of the second process in terms of an association according to equation (1). Nevertheless, the inverse of the micellar association constants obtained in this way provide an objective measure of the concentrations of surfactant at which the absorbance changes associated with the various equilibrium processes are half-complete.

Fig. 5 shows the effect of the concentration of deuteroferrihaem on the initial rate of reaction with *m*-chloroperoxybenzoic acid. The previous treatment[6] for the kinetics at constant haem concentration used equation (4) which relates k_1,

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

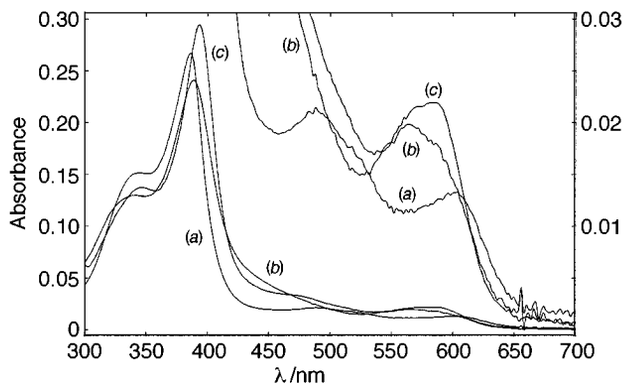

Fig. 3 Spectrum of 3.08×10^{-6} mol dm^{-3} deuteroferrihaem in (*a*) pH 7.4 buffer alone and in buffer containing 1×10^{-3} (*b*) and 6×10^{-2} mol dm^{-3} Triton X-100 (*c*)

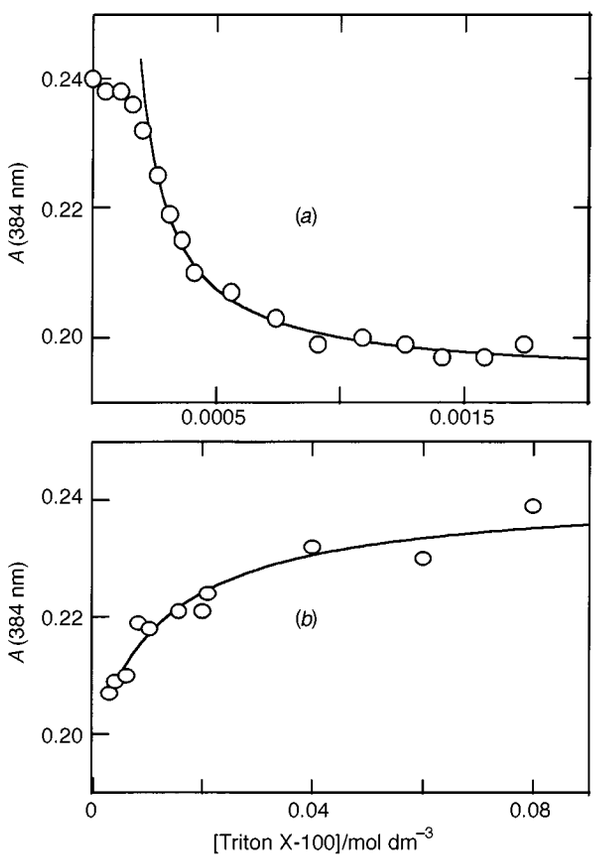

Fig. 4 Effect of Triton X-100 concentration, (*a*) lower and (*b*) higher range, on the absorbance of deuteroferrihaem at 384 nm; conditions as Fig. 3. The two curves represent equation (3) with the two sets of best-fit parameters

the rate-limiting second-order rate constant, to the initial rate of absorbance change, the overall stoichiometric number, *i.e.* 4, the total concentration of peroxy acid, $[HO_2X]_0$, *i.e.* protonated plus anionic forms (not just the protonated form as in the erroneous statement in ref. 6), ΔA_{max}, the absorbance change for the complete conversion of haem into the long-lived intermediate and α_0, the degree of dissociation of the haem calculated from the dimerization constant, K_D, at the initial haem concentration. Substituting into equation (4) the relationships in equations (5) and (6), where $\Delta\varepsilon$ is the difference in absorption

$$\Delta A_{max} = \Delta\varepsilon[FeP]_0 \quad (5)$$

$$[\text{haem monomer}] = \alpha_0[FeP]_0 \quad (6)$$

coefficients of the haem and the intermediate, and $[FeP]_0$ is the

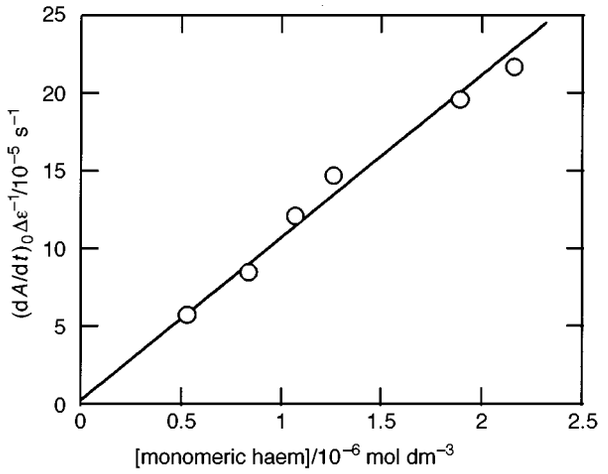

Fig. 5 Initial first-order rate constant for the reaction in pH 7.4 buffer between (1–10) × 10^{-6} mol dm^{-3} deuteroferrihaem and 3.0 × 10^{-6} mol dm^{-3} *m*-chloroperoxybenzoic acid as a function of the concentration of haem monomer

total concentration of haem, leads to equation (7). The

$$k_1 = \frac{-(\mathrm{d}A/\mathrm{d}t)_0}{4[\mathrm{HO_2X}]_0\Delta\varepsilon[\text{haem monomer}]} \quad (7)$$

concentration of haem monomer in Fig. 5 is calculated using $[\mathrm{FeP}]_0$ and the value of K_D, 8.54 × 10^5 dm^3 mol^{-1} calculated at pH 7.4 from ref. 3. The linear relationship, with negligible intercept, in Fig. 5 confirms the validity of K_D under the present experimental conditions and the validity of a rate-limiting step involving haem monomer. The gradient yields a value of k_1, according to equation (7), of 8.7 × 10^6 dm^3 mol^{-1} s^{-1} virtually identical to that reported previously.[6]

In the presence of sds or Triton X-100 the initial rate of reaction of 3.04 × 10^{-6} mol dm^{-3} deuteroferrihaem with *m*-chloroperoxybenzoic acid is first order with respect to peroxy acid concentration in the range 2.5 × 10^{-7} to 1.5 × 10^{-5} mol dm^{-3} (results not shown). Observed second-order rate constants, calculated from the initial rate data according to equation (8), which differs from (4) by the absence of α_0, at

$$k_{obs} = \frac{-(\mathrm{d}A/\mathrm{d}t)_0}{4[\mathrm{HO_2X}]_0\Delta A_{max}} \quad (8)$$

various concentrations of sds are in Table 1 and of Triton X-100 in Table 2.

We have recently treated the kinetics of bimolecular reactions in aqueous surfactant solutions using a multiple micellar pseudo-phase (MMPP) model according to equation (9) where

$$k_{obs} = \frac{k_w + (k_{mic} - k_w\bar{V}_{mic})([S] - \text{c.m.c.})}{\{1 + (K^A_{mic} - \bar{V}_{mic})([S] - \text{c.m.c.})\} \times \{1 + (K^B_{mic} - \bar{V}_{mic})([S] - \text{c.m.c.})\}} \quad (9)$$

k_{obs} is the observed second-order rate constant, k_w the rate constant in the bulk aqueous phase, k_{mic} an observed third-order rate constant (first order in surfactant concentration), $\bar{V}_{mic}$ the effective molar volume of micellized surfactant, and the micellar association constants, K^A_{mic} and K^B_{mic}, approximate to K_A and K_B respectively [defined in equations analogous to (1) and (2) for R] provided that the actual volume of the micellar pseudo-phase is small compared with the volume of the bulk aqueous phase.[8] Application of the transition-state pseudo-equilibrium constant approach to the MMPP model leads to the relationship (10) where the micellar association constant of

$$k_{mic}/k_w = K^{TS}_{mic} \quad (10)$$

Table 1 Second-order rate constants (mean ± standard deviation of runs at more than ten different peroxy acid concentrations) for the reaction of deuteroferrihaem and *m*-chloroperoxybenzoic acid in pH 7.4 buffered sds

10^3 [sds]/mol dm^{-3}	10^{-6} k_{obs}/dm^3 mol^{-1} s^{-1}
0.56	4.1 ± 0.2
1.81	3.6 ± 0.2
5.0	3.05 ± 0.13
10.0	2.05 ± 0.08
20.0	1.16 ± 0.02
40	0.63 ± 0.01
65	0.45 ± 0.01
100	0.313 ± 0.005
150	0.210 ± 0.003

Table 2 Second-order rate constants (mean ± standard deviation of runs at generally more than ten different peroxy acid concentrations) for the reaction of deuteroferrihaem and *m*-chloroperoxybenzoic acid in pH 7.4 buffered Triton X-100

10^3 [Triton X-100]/mol dm^{-3}	10^{-6} k_{obs}/dm^3 mol^{-1} s^{-1}
0.150	2.25 ± 0.05
0.35	1.18 ± 0.13
0.88	0.85 ± 0.10
1.16	0.78 ± 0.08
5.0	0.45 ± 0.03
12.5	0.255 ± 0.015
20.0	0.220 ± 0.013
40	0.283 ± 0.008

the transition state, K^{TS}_{mic}, is again defined according to equations (1) and (2) and represents the stabilization of the transition state by the micelle.[8] Combining equations (9) and (10) leads to (11) which shows how the quotient k_{obs}/k_w relates to the micellar

$$\frac{k_{obs}}{k_w} = \frac{1 + (K^{TS}_{mic} - \bar{V}_{mic})([S] - \text{c.m.c.})}{\{1 + (K^A_{mic} - \bar{V}_{mic})([S] - \text{c.m.c.})\} \times \{1 + (K^B_{mic} - \bar{V}_{mic})([S] - \text{c.m.c.})\}} \quad (11)$$

association constants of the transition state and reactants. Thus equation (11) describes the stabilization of the transition state and the reactants by the surfactant micelles relative to their stability in the bulk aqueous phase under the experimental conditions. In order to consider the stabilization of the transition state by the micelles with respect to the stability of the transition state formed from the reaction of monomeric haem in water, k_w in equation (11) is equated with k_1 in (4) to give equation (12), in which the micellar association constants of the

$$\frac{k_{obs}}{k_1} = \frac{1 + (K^{TS}_{mic} - \bar{V}_{mic})([S] - \text{c.m.c.})}{\{1 + (K^{mcpba,\ pH\ 7.4}_{mic} - \bar{V}_{mic})([S] - \text{c.m.c.})\} \times \{1 + (K^{haem,\ pH\ 7.4}_{mic} - \bar{V}_{mic})([S] - \text{c.m.c.})\}} \quad (12)$$

haem and *m*-chloroperoxybenzoic acid under the experimental conditions, pH 7.4, are written as $K^{haem,\ pH\ 7.4}_{mic}$ and $K^{mcpba,\ pH\ 7.4}_{mic}$ respectively. Values of $K^{mcpba,\ pH\ 7.4}_{mic} - \bar{V}_{mic}$ are calculated using the corresponding micellar association constant terms of the conjugate acid and base forms of the peroxy acid, $K^{PH}_{mic} - \bar{V}_{mic}$ and $K^{P}_{mic} - \bar{V}_{mic}$, obtained from the pH dependence of the micelle-mediated reaction of *m*-chloroperoxybenzoic acid and iodide, according to equation (13) where K_a is the mixed acid-

$$K^{mcpba,\ pH}_{mic} - \bar{V}_{mic} = \frac{\{H^+\}_w(K^{PH}_{mic} - \bar{V}_{mic}) + K_a(K^{P}_{mic} - \bar{V}_{mic})}{\{H^+\}_w + K_a} \quad (13)$$

dissociation constant of the peroxy acid and $\{H^+\}_w$ is the hydrogen-ion activity measured with the glass electrode.[8]

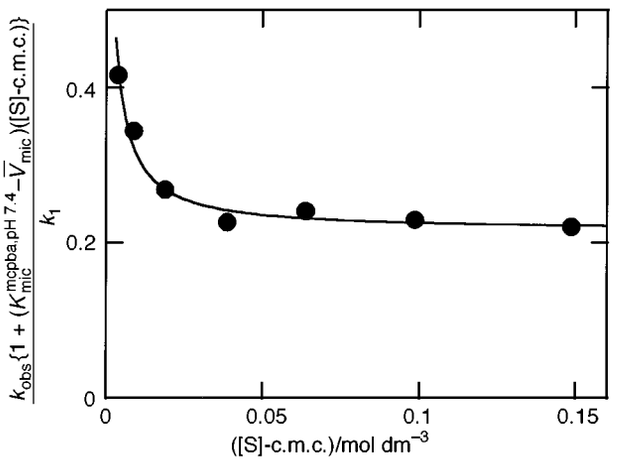

Fig. 6 Effect of sds on the reaction of deuteroferrihaem and *m*-chloroperoxybenzoic acid. Data treated according to equation (14) with the micellar association constant term, $K_{mic}^{mcpba,\ pH\ 7.4} - \bar{V}_{mic}$, 56 dm^3 mol^{-1}; the curve represents the equation with the best-fit values of $K_{mic}^{TS} - \bar{V}_{mic}$ and $K_{mic}^{haem,\ pH\ 7.4} - \bar{V}_{mic}$

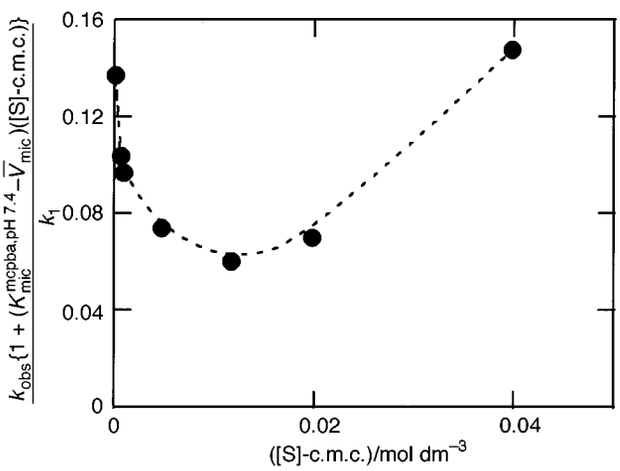

Fig. 7 Effect of Triton X-100 on the reaction of deuteroferrihaem and *m*-chloroperoxybenzoic acid. Data treated according to equation (14) with the micellar association constant $K_{mic}^{mcpba,\ pH\ 7.4} - \bar{V}_{mic}$, 89 dm^3 mol^{-1}; the dashed curve is merely a guide for the eye

Using a pK_a value of 7.53 for *m*-chloroperoxybenzoic acid[10] and $(K_{mic}^{PH} - \bar{V}_{mic})$ and $(K_{mic}^{P} - \bar{V}_{mic})$ 152 and 4.1 dm^3 mol^{-1}, respectively, in Triton X-100, and 94 dm^3 mol^{-1} and zero, respectively, in sds, gives values of $K_{mic}^{mcpba,\ pH\ 7.4} - \bar{V}_{mic}$, 89 and 56 dm^3 mol^{-1} in Triton X-100 and sds, respectively.[8] These values are appropriate for the current work if it is assumed that association of deuteroferrihaem with the micelles does not affect their affinity for the peroxy acid. Equation (12) is rearranged to (14)

$$\frac{k_{obs}\{1 + (K_{mic}^{mcpba,\ pH\ 7.4} - \bar{V}_{mic})([S] - \text{c.m.c.})\}}{k_1} = \frac{1 + (K_{mic}^{TS} - \bar{V}_{mic})([S] - \text{c.m.c.})}{1 + (K_{mic}^{haem,\ pH\ 7.4} - \bar{V}_{mic})([S] - \text{c.m.c.})} \quad (14)$$

and Fig. 6 shows a plot of the left-hand side of equation (14) against ([sds] − c.m.c.) for the data in Table 1, with the omission of the first two entries where the [sds] is below or very close to the c.m.c. Fitting equation (14) to the data in Fig. 6 leads to the best-fit values $K_{mic}^{TS} - \bar{V}_{mic}$ 150 ± 20 dm^3 mol^{-1} and $K_{mic}^{haem,\ pH\ 74} - \bar{V}_{mic}$ 720 ± 80 dm^3 mol^{-1}, the latter in good agreement with the value of 870 ± 100 dm^3 mol^{-1} obtained from the effect of sds on the absorbance of deuteroferrihaem at 396 nm shown in Fig. 2. Fig. 7 shows a plot of the left-hand side of equation (14) against ([Triton X-100] − c.m.c.) for the data in Table 2, with the omission of the first entry where the [Triton X-100] is below the c.m.c. A qualitative agreement is observed between these kinetic data and the effect of Triton X-100 on the absorbance of deuteroferrihaem at 384 nm shown in Fig. 4.

$$\begin{array}{ccc} 2[FeP]^+ & \rightleftharpoons & [(FeP)_2OH]^+ + H^+ \\ \uparrow\downarrow & & \uparrow\downarrow \\ 2H^+ + 2[FeP(OH)] & \rightleftharpoons & [(FeP)_2O] + 2H^+ \end{array}$$

Scheme 1

Discussion

Only a few studies of the interaction of deuteroporphyrinatoiron complexes and surfactant micelles have been reported and to our knowledge these involve either the pyridine complex or the iron(II) oxidation state.[11] There are, however, studies of protoferrihaem in micelles that are pertinent to the present work. A Mössbauer study of protoferrihaem in sds has been described and the electronic absorption spectrum compared with those of tetraphenylporphyrin complexes of iron(III).[12] Proton and ^{13}C NMR studies indicate that protoferrihaem complexes are located close to the surface of the sds micelle.[13] In agreement with the present results concerning deuteroferrihaem, it is reported that the c.m.c. does not show any drastic change on association of the complexes and this is consistent with the micelles retaining their shape.[13b]

The changes reported in the Soret region on addition of surfactants to protoferrihaem are attributed to haem monomerization and are much more pronounced than those in the present work with deuteroferrihaem.[14] This is because protoferrihaem is almost completely dimeric in the absence of surfactant whilst a significant proportion of deuteroferrihaem is already in the form of monomer under the present experimental conditions.[3] Scheme 1 shows some of the possible ionization and dimerization equilibria for ferrihaems, $[FeP]^+$, where P is the porphyrinato dianion; charges on ionized propionic acid ring substituents are ignored, and axial water ligands are omitted.

The dimerization constant of deuteroferrihaem is directly proportional to the hydrogen-ion concentration between pH 7 and 8, consistent with the equilibrium between $[FeP]^+$ and the μ-hydroxo-bridged dimer $[(FeP)_2OH]^+$ shown in Scheme 1.[3] It is worth noting that in addition to the differences in reactivity with peroxides between protoferrihaem derivatives and synthetic ferrihaems that we have previously considered,[6] there are, in many cases, significant differences in ionization and dimerization behaviour.[3,15] A typical equilibrium is for tetrakis(4-sulfonatophenyl)porphyrinatoferrate(III) and involves $[FeP]^+$ and $[(FeP)_2O]$ so that the dimerization constant is proportional to the square of the hydrogen-ion concentration.[16] Appropriately hindered porphyrins such as tetrakis(2,6-dimethyl-3-sulfonatophenyl)porphyrinatoferrate(III) and an octacationic tetraphenylporphyrinato iron(III) do not undergo dimerization upon ionization.[17] Notwithstanding this, the dimerization constant for deuteroferrihaem reported in ref. 3 gives a concentration-dependent pK_a of 7.0 when the total haem concentration is 3.08 × 10^{-6} mol dm^{-3}. Hence the different spectra in the presence of sds (Fig. 1) and Triton X-100 (Fig. 3) at pH 7.4 are probably due to differences in the ionization of the haem, since surfactants can change the apparent pK_a of associated acids by as much as 1.5 units.[8] The negatively charged sds micelle and the consequent raised hydrogen-ion concentration close to its surface, relative to the bulk aqueous phase, is likely to stabilize the protonated form of the haem with the highest charge density, *i.e.* $[FeP]^+$. On the other hand, a non-ionic surfactant because of the greater energy required for charge separation in the lower relative permittivity environment of the micelle is likely to favour neutral complexes such as [FeP(OH)] or $[(FeP)_2O]$. These factors have been discussed for the effect of

the different surfactants on the pK_a of pyridine complexes of deuteroferrihaem.[11a] The spectra in Figs. 1 (sds) and 3 (Triton X-100 at the higher concentration) respectively resemble those of protoferrihaem in sds at pH 2.6 and 12.5,[13a] which is further evidence for our assignment of aqua and hydroxo ligands to the deuteroferrihaem in the respective surfactants. On the basis of charge considerations, the hydroxo form of deuteroferrihaem and the μ-oxo dimer are equally favoured in Triton X-100. Bearing in mind, however, the strength of the first association process shown in Fig. 4, the haem is concentrated in a relatively small volume of surfactant pseudo-phase and this concentration would favour the μ-oxo dimer form. The subsequent change of the Soret λ_{max} to longer wavelengths with increasing amounts of Triton X-100 (Fig. 3) is consistent with monomerization as the haem becomes diluted in the surfactant phase.

In the results section the kinetics is treated using the multiple micellar pseudo-phase (MMPP) model[8] according to equation (14) where the value of the micellar association constant term of the peroxy acid, $K_{mic}^{mcpba,\ pH\ 7.4} - \bar{V}_{mic}$ is taken from an independent kinetic study under comparable conditions.[8] This is justified as follows. According to the MMPP model the reactants partition between the bulk water phase and an arbitrary number of micellar pseudo-phases, each with different partition coefficients for the reactants and a different rate constant. The sum of the rates of the concurrent reactions in the various phases constitutes the overall rate. The model is valid for cases where a reactant associates with one region of the micelle but undergoes reaction in an adjacent region where it has lower affinity. The micellar association constants obtained from the model are overall values reflecting the total amount of reactant binding and should be independent of the nature of the reaction partner, provided the latter does not change the nature of the micelles. In contrast to this, of course, the micellar association constant of the transition state is dependent on the affinity of the reaction partner for the particular region of the micelle where the majority of the reaction takes place.

The micellar association constant terms from the data treatment include the negative of the effective molar volume of micellized surfactant, $\bar{V}_{mic}$. These values are estimated at 0.4 and 2.1 $dm^3\ mol^{-1}$ for sds and Triton X-100, respectively,[8] and are small compared with the parameters obtained from the curve fitting and so are subsequently neglected. The value of the micellar association constant of the ferrihaem, $K_{mic}^{haem,\ pH\ 7.4}$, 720+80 $dm^3\ mol^{-1}$, obtained from the kinetics in sds is in good agreement with the value of 870 ± 100 $dm^3\ mol^{-1}$ obtained from the effect of sds on the absorbance of deuteroferrihaem at 396 nm. This confirms the assumption that the association of the deuteroferrihaem with sds does not significantly affect the interaction of the micelle with *m*-chloroperoxybenzoic acid. The value of the micellar association constant of the transition state, K_{mic}^{TS}, 150 ± 20 $dm^3\ mol^{-1}$ reflects the stabilization of the transition state in the sds micelle compared with that involving the monomeric deuteroferrihaem in the aqueous buffer. It is significantly less than $K_{mic}^{haem,\ pH\ 7.4}$ and this shows that the sds micelle stabilizes the haem more than the transition state and hence the kinetic data represent micellar inhibition. If, as is almost certainly the case, the transition state involves one less proton than the reactants,[4,5] then the relative destabilization of the transition state is due to the high local hydrogen-ion concentration at the surface of the negatively charged sds micelle.

The kinetic data in Triton X-100 shown in Fig. 7 are qualitatively similar to the spectroscopic data shown in Fig. 4. We were unable to obtain unique solutions to equations representing possible reaction schemes, however, since the absorption coefficients of micelle-associated species could not be assigned unambiguously; similar problems have been encountered in treating data concerning the transfer of protoferrihaem from cytochrome b_5 to dicetyl phosphate vesicles *via* an intermediate haemprotein species.[18] Data treatment may be further complicated by the possible saturation of the Triton X-100 micelles, aggregation number 100,[19] by the haem at the lower surfactant concentrations. Nevertheless, equation (14) can be safely approximated by (15) because of the high values of the associ-

$$\frac{k_{obs}\{1 + (K_{mic}^{mcpba,\ pH\ 7.4} - \bar{V}_{mic})([S] - \text{c.m.c.})\}}{k_1} = \frac{K_{mic}^{TS}}{K_{mic}^{haem,\ pH\ 7.4}} \quad (15)$$

ation constants on the right-hand side of the equations. Hence, according to equation (15) the points at the higher surfactant concentrations in Fig. 7 represent the increase in transition-state stabilization with respect to the stabilization of the deuteroferrihaem, $K_{mic}^{TS}/K_{mic}^{haem,\ pH\ 7.4}$, from 0.06 to 0.14 as the Triton X-100 concentration increases. This is consistent with the monomeric haem species being the reactive form in the micelle, as it is in water.

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