Equilibrium and Kinetic Studies of Deuteroferrihaem {Chloro[dihydrogen-3,7,12,17-tetramethyl-2,18-porphinedipropionato(2−)]iron(III)} Dimerization

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The dimerization constant of deuteroferrihaem (K = [Dimer][H$^+$]/[Monomer]^2) at 25 °C and I = 0·1 M has been redetermined as (3·4 ± 0·2) × 10^{-2}. Studies of the temperature dependence of the equilibrium (range 5—25 °C) yield ΔH^0 = +44 kJ mol^{-1} and ΔS^0 (at 298 K) = +120 J K^{-1} mol^{-1}. The kinetics of monomer-dimer interconversion have been examined by temperature-jump spectrophotometry over a limited pH range at 25 °C and I = 0·1 M. At pH 6·70 the dimerization rate constant k_f = 2·6 × 10^8 l mol^{-1} s^{-1} and the dissociation rate constant k_r = 1·1 × 10^3 s^{-1}; at pH 7·05, k_f = 3·9 × 10^8 l mol^{-1} s^{-1} and k_r = 0·7 × 10^3 s^{-1}.

EQUILIBRIUM data for the dimerization of deuteroferrihaem (dfh) † and protoferrihaem (pfh) have previously been reported.[1] The role of dimerization in the catalytic action of ferrihaem compounds has been discussed and comparative studies of ferrihaem catalysis of hydrogen peroxide decomposition with the behaviour of the enzyme catalase (E.C. 1.11.1.6.) have been made.[2-4] The present paper reports further studies of the dimerization equilibrium of dfh at 25 °C, an examination of the effect of temperature on this equilibrium in the range 5—25 °C, and studies of the kinetics of interconversion of monomeric and dimeric dfh.

EXPERIMENTAL

The preparation of materials and solutions followed the procedures described previously.[1] In all experiments the phosphate buffer (Na_2HPO_4–KH_2PO_4) concentration was 0·01 mol l^{-1} and the ionic strength was maintained at 0·1 mol l^{-1} by addition of sodium chloride. Primary solutions were prepared from accurately weighed amounts of deuteroferrihaem (dfh) and, from these, more dilute solutions were prepared by quantitative dilution.

Equilibrium Studies.—The dimerization equilibrium was studied spectrophotometrically using a Unicam SP 1800 spectrophotometer. Computations were made using the measured absorption coefficient at 384 nm of dfh solutions (concentration range *ca.* 10^{-7}—10^{-4} mol l^{-1}). Cuvettes with path lengths in the range 0·1—10 cm were employed, and, except for 10 cm cells, the temperature was controlled by circulating aqueous glycol from a Grant LB 34 refrigerated thermostat through the cuvette jacket. The temperature of solutions was measured using a thermocouple immersed in the solution but out of the light path. A cuvette jacket was not available for 10 cm cells and in this case solutions were pre-thermostatted in an air thermostat. The cell was then surrounded, except for the windows, with closely fitting insulation, transferred to the spectrophotometer, and measurements made as rapidly as possible.

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† *Nomenclature*:—Iron porphyrin complexes are described in the literature by a variety of trivial names. In this paper the trivial names used are those recommended by the International Union of Biochemistry (' Enzyme Nomenclature,' Elsevier, 1965, p. 19). Deuteroferrihaem (dfh) is the complex chloro[dihydrogen-3,7,12,17-tetramethyl-2,18-porphinedipropionato(2−)]iron(III) (*Chem. Abs.* Index No. 18922-88-8).

[1] S. B. Brown, T. C. Dean, and P. Jones, *Biochem. J.*, 1970, **117**, 733.

[2] S. B. Brown, T. C. Dean, and P. Jones, *Biochem. J.*, 1970, **117**, 741.

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[4] P. Jones in ' Oxidases and Related Redox Systems,' eds. T. E. King, H. S. Mason, and M. Morrison, Baltimore, University Park Press, 1973.

Kinetic Studies.—The kinetics of dfh monomer–dimer interconversion were studied by temperature-jump relaxation spectrophotometry using a Messanlangen Studiengesellschaften type SBA7 instrument. The temperature jump employed was from 22·4 to 25 °C. Some preliminary experiments were carried out using a Durrum D-150 instrument.

RESULTS

Dimerization Equilibrium.—The general form of the results at 25 °C confirmed the observations of previous work.[1] The variation of the observed stoicheiometric absorption coefficient (at 384 nm), ε_{obs}, with deuteroferrihaem concentration, [dfh], at several pH values is illustrated in Figure 1. The full curves shown in Figure (1*a*) derive from a computer fit obtained using the following procedure.

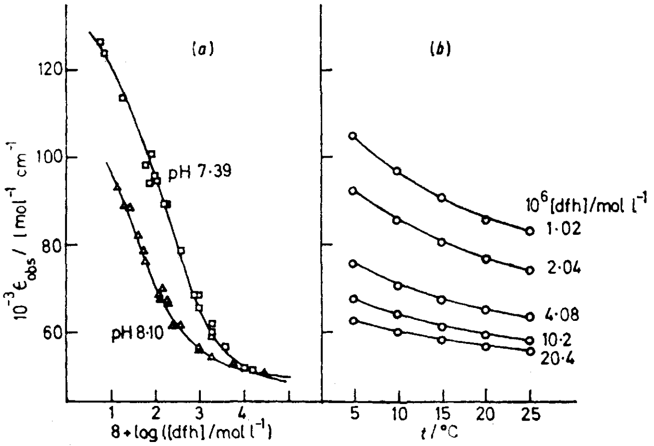

FIGURE 1 Dependence of ε_{obs} on: (*a*) deuteroferrihaem concentration, [dfh], and pH at 25 °C; and (*b*) [dfh] and temperature at pH 7·75

Adopting the notation used previously,[1] the dimerization process may formally be represented by equation (1) and we

$$2M \overset{K}{\rightleftharpoons} D + nH^+ \qquad (1)$$

define the observed dimerization constant at fixed pH as in (2). It may be shown that expression (3) is applicable, where

$$K_{obs} = K/[H^+]^n \qquad (2)$$

$$K_{obs} = (1 - \alpha)/2\alpha^2[\text{dfh}] \qquad (3)$$

α is the fraction of dfh in the form of the monomer. The observed absorption coefficient at fixed wavelength and pH is then given by (4) (ε_M is the absorption coefficient of

$$\varepsilon_{obs} = A\varepsilon_M + B(\varepsilon_D/2) \qquad (4)$$

where

$$A = \frac{(1 + 8[\text{dfh}]K_{obs})^{\frac{1}{2}} - 1}{4[\text{dfh}]K_{obs}}$$

and $B = 1 - A$

monomeric ferrihaem and ε_D that of dimeric ferrihaem under the prescribed conditions). Thus lim([dfh] $\longrightarrow$ 0), $A \longrightarrow 1$ and $\varepsilon_{obs} \longrightarrow \varepsilon_M$, and lim ([dfh] $\longrightarrow \infty$), $A \longrightarrow 0$ and $\varepsilon_{obs} \longrightarrow \varepsilon_D/2$.

Initially, experimental data were smoothed by eye in order to obtain equispaced data points and so avoid weighting errors derived from the experimental procedure. These data were processed using a non-linear least-squares program which yielded the best fit to equation (4). The maximum deviation between computed curves and smoothed data was 2%. Values of K_{obs} obtained are plotted in Figure 2 and confirm the previous result that, in equation (1), $n = 1$. From these results the dimerization constant K at 25 °C and $I = 0{\cdot}1$M was $(3{\cdot}4 \pm 0{\cdot}2) \times 10^{-2}$ (compared with $1{\cdot}9 \times 10^{-2}$ obtained previously by graphical procedures). At other temperatures α was calculated from equation (5), whence K_{obs} was obtained, using the relation

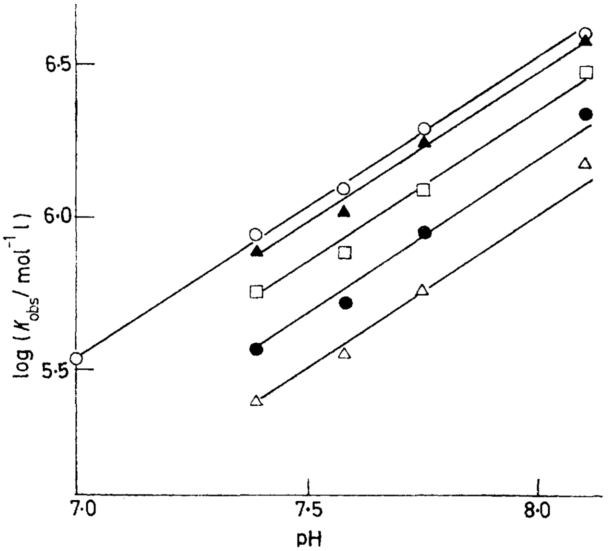

FIGURE 2 pH Dependence of K_{obs} at 25 (○), 20 (▲), 15 (□), 10 (●), and 5 °C (△)

$$\alpha = \frac{\varepsilon_{obs} - (\varepsilon_D/2)}{\varepsilon_M - (\varepsilon_D/2)} \qquad (5)$$

$$\alpha^2[\text{dfh}] = \frac{1}{2\,K_{obs}} - \frac{1}{2\,K_{obs}}\,\alpha \qquad (6)$$

(6). The temperature dependencies of ε_M and ε_D were assumed to be negligible over the range considered. For

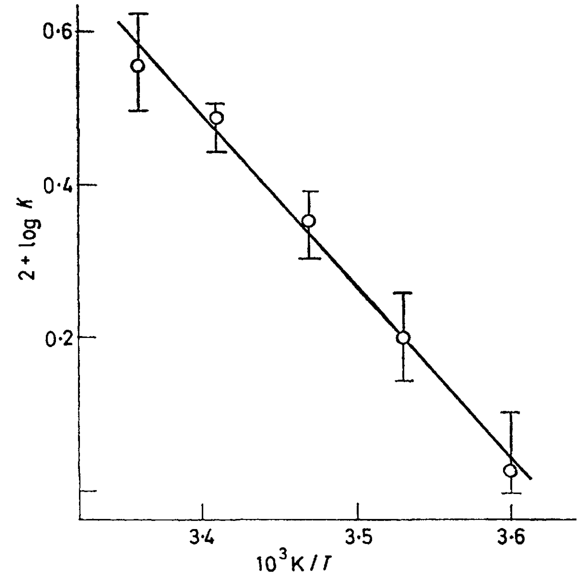

FIGURE 3 Temperature dependence of the dimerization constant K

ε_D this assumption was checked by demonstrating that, at fixed pH, plots of ε_{obs} against $[dfh]^{-\frac{1}{2}}$ at high [dfh] extrapolated to a constant value within experimental error at $[dfh]^{-\frac{1}{2}} = 0$. A similar test for ε_M could not be made with the data available, but temperature-jump experiments suggest that the variation is small. On this basis, the K_{obs} values shown in Figure 2 were obtained. Derived values of K at different temperatures are plotted in Figure 3 and this treatment yields the following thermodynamic data for the dimerization equilibrium: $\Delta H^0 = +44$ kJ mol^{-1}; ΔS^0 (25 °C) $= +120$ J K^{-1} mol^{-1}.

Kinetics of Monomer–Dimer Interconversion.—The kinetics of deuteroferrihaem monomer–dimer interconversion were examined by the temperature-jump method using spectrophotometric detection at 384 nm. In all experiments a single relaxation was observed. This was preceded by an unresolved fast (<10 μs) process of small amplitude, which is probably related to a rapid monomer-protonation equilibrium.[1] Figure 4 shows that the concentration dependence of the relaxation times at pH 6·70 and 7·05 agrees well (correlation coefficients >0·98) with the expectation for a dimerization process {equation (7), where $[M_T]$ is the total monomer concentration}. From the plots shown in Figure 4 the following results were obtained:

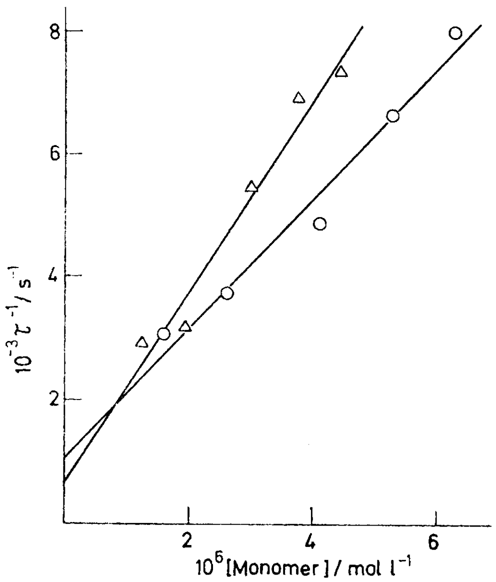

FIGURE 4 Variation of τ^{-1} with concentration of dfh monomer at pH 7·05 (△) and 6·70 (○)

$$\tau^{-1} = 4k_f[M_T] + k_r \qquad (7)$$

	$10^{-8}k_f$	$10^{-3}k_r$	log K_{obs}	
pH	l mol^{-1} s^{-1}	s^{-1}	calc.	expt.
6·70	2·6	1·1	5·4	5·2
7·05	3·9	0·7	5·7	5·6

The pH range over which measurements could be made was severely limited. A lower limit is set by solubility and an upper limit by the decrease in relaxation amplitude with increasing pH. We also carried out experiments at pH 7·50, which yielded relaxation times comparable to those at lower pH and a similar trend of relaxation time with monomer concentration, but the scatter is considerable and the correlation coefficient was 0·93 in plots according to equation (7). These restrictions preclude a detailed path analysis, although the relative insensitivity of k_f and k_r to pH, and the value of $pK_{a(M)}$, suggest that the major path under the conditions of our experiments is probably $M + M' \rightleftharpoons D$, where M′ is the conjugate base [1] of M.

DISCUSSION

A major objective of the present work was to obtain background energetic and kinetic information required for analysis of both steady-state and pre-steady-state experiments on the catalytic properties of deuteroferrihaem (dfh). The substantial difference in dimerization constant between dfh and protoferrihaem (pfh) has been useful in examining the role of dimerization in the catalytic properties of these species.[2,3] Preliminary studies of the aggregation of corresponding metal-free porphyrins suggest that their dimerization constants are comparable with the ferrihaem compounds and imply that the differences originate primarily in interactions at the periphery of the porphyrin ring. It is interesting to note that the required difference in free energy of dimerization ($\Delta G_{dfh}{}^0 - \Delta G_{pfh}{}^0 = -11{\cdot}8$ kJ mol^{-1}) is comparable to the contribution which might be expected from pairwise hydrophobic interactions [5] of vinyl side chains which are present in the protoporphyrin and pfh species. Protoferrihaem solutions are so highly dimeric that it is improbable that satisfactory studies of the energetics of dimerization of this species could be made. For the same reason, temperature-jump studies of pfh solutions show no discernible relaxation processes. Our kinetic experiments show that, at least at low pH, the assumption of dimerization pre-equilibrium is valid both in interpretation of steady-state catalytic kinetics [2,3] and analysis of pre-steady-state studies of the function of catalytic intermediates.[6] The implication of the results in the latter context will be discussed in detail elsewhere.[7]

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[7] P. Jones and H. C. Kelly, unpublished work.