Interaction of Surfactant-solubilized Monomeric Hemin with Imidazole in Water, Cyclohexane, and Benzene: Effects of Aqueous and Reversed Micelles

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Hemin is present as monomers in aqueous micellar hexadecyltrimethylammonium bromide (htab), and sodium dodecyl sulphate, Na(ds), as well as in polar solvent pools in cyclohexane and in benzene, solubilized by polyoxyethylene (6) nonylphenol, Igepal CO-530. Interaction of imidazole (im) with the surfactant-solubilized monomeric hemin results in the equilibrium formation of bis(imidazole)hemin. Equilibrium constants for the formation of this complex, $K_{app.}$, have been determined in aqueous 0.05 mol dm^{-3} htab and 0.50 mol dm^{-3} Na(ds) as functions of pH. These data have been rationalized in terms of two kinetically indistinguishable mechanisms: hemin(OH^-, $H_2O)_2$(S) + imH^+ + im $\overset{K'}{\rightleftharpoons}$ hemin$(im)_2$ + $2H_2O$; and hemin$(H_2O)_2$(S) + 2im $\overset{K''}{\rightleftharpoons}$ hemin$(im)_2$ + $2H_2O$. Values of the pH-independent equilibrium constants, K' and K'', have been calculated in 0.05 mol dm^{-3} htab as 1.2×10^5 and 1.0×10^7 and in 0.50 Na(ds) as 1.0×10^6 and 5.0×10^9 dm^6 mol^{-2}. These should be compared with values of $K' = 8.0 \times 10^6$ dm^6 mol^{-2} and $K'' = 4.0 \times 10^7$ dm^6 mol^{-2} reported in ethanol–water (8.9 : 11.1 w/w). Equilibrium constants for the formation of bis(imidazole)hemin have also been determined in methanol and water pools, solubilized by Igepal CO-530 in cyclohexane and in benzene, together with rate constants for the equilibrium attainment of the complex. Evidence has been deduced for the two-step addition of imidazole to hemin. Micellar effects on this reaction are discussed in terms of selective substrate concentration, electrostatic interactions, and alterations of the effective dissociation constants and of the microenvironments of the reactants. The reactions of imidazole and cyanide ions on hemin micellar solutions are compared.

THE iron porphyrin moiety is the reactive centre of such biologically important molecules as haemoglobins, myoglobins, catalases, peroxidases, and cytochromes.[1a-g] The importance of imidazole co-ordination (as histidines) to the iron biosphere has prompted numerous investigations on the interaction of iron porphyrins and related compounds with imidazole.[1-15] Although these studies have provided much information, effects of changes in the microenvironment on the thermodynamic parameters for imidazole binding have scarcely been examined. In water, hemin (iron protoporphyrin) is dimeric. Conversely, it is monomeric in alcohol–water mixtures,[11-13] in dimethyl sulphoxide (dmso),[14a,16] and in aqueous [2,17-19] and non-aqueous micelles.[15] The use of a micellar surfactant allows, therefore, the kinetic investigation of substitution reactions with hemin monomers. The present paper reports kinetic and thermodynamic studies on the interaction of imidazole with surfactant-solubilized hemin in water, cyclohexane, and benzene.

EXPERIMENTAL

Hemin (ferriprotoporphyrin IX) was used as received from Eastman. Its purity was established from absorption spectrophotometry. Polyoxyethylene(6) nonylphenol (Igepal CO-530) was used as received from GAF Corporation. Due to their preparation, different batches of Igepal CO-530 were found to contain differing amounts of base contaminants. Since the concentration of base can affect the results, the amount present in each batch was determined by titration with standard 0.010 mol dm^{-3} HCl (B.D.H.) using lacmoid indicator. The base in Igepal CO-530 ranged from 2.2×10^{-3} mol dm^{-3} in lot 206 to 5.0×10^{-4} mol dm^{-3} in lot 219. Therefore, the base concentrations were adjusted to the desired value before any kinetic and equilibrium determinations were made using Igepal CO-530. Thus, duplicate kinetic and equilibrium determinations using different batches of Igepal CO-530 gave the same results within experimental error. Reagent-grade imidazole (Aldrich) was dried *in vacuo* over P_4O_{10} before use and its purity was established by melting-point determination and by its ^1H n.m.r. spectra. The surfactants, hexadecyltrimethylammonium bromide (htab) and sodium dodecyl sulphate, Na(ds), were prepared and

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purified using established methods.[20] Reagent-grade benzene was distilled from sodium and stored over Linde type 4A molecular sieves. Anhydrous methanol (Fisher) and cyclohexane (Matheson, Coleman, and Bell) were further dried with activated type 4A molecular sieves for several days before use. The water content of the organic solvents used was carefully controlled and monitored by use of gas–liquid partition chromatography employing a Porapak Q column (Water Associates).[21] All the other chemicals used were the best available reagent-grade materials.

Stock solutions of hemin (generally 10^{-3} mol dm^{-3}) in methanol were made up under a stream of dry nitrogen in dry 0.03 mol dm^{-3} methanolic sodium methoxide and stored in the refrigerator. These solutions were usually stable for at least 2 weeks as monitored by absorption spectrophotometry. Stock solutions of hemin in distilled water were generally prepared under dry nitrogen immediately prior to use by dissolving known amounts of solid hemin in aqueous sodium hydroxide followed by volumetric dilution. In all cases, the final hemin concentrations were determined from absorption spectrometry by employing a calibration graph.

Individual solutions for the spectral and kinetic determinations were prepared by injecting appropriate volumes of the stock hemin solutions into appropriate surfactants. Final concentrations of hemin ranged between 5×10^{-6} and 1×10^{-5} mol dm^{-3}. Imidazole solutions were prepared by weighing out known amounts of the dry imidazole and diluting to final volume using the appropriate surfactant–solvent system. Final imidazole concentrations usually ranged from 1.0×10^{-4} to 9.0×10^{-2} mol dm^{-3}. Equilibrium and kinetic determinations were carried out under pseudo-first-order conditions, *i.e.* [imidazole] $\gg$ [hemin].

The final bis(imidazole)hemin product was also independently synthesized using a slightly modified procedure to that described in the literature.[22] The absorption spectral characteristics of this isolated product were virtually identical to that of the product generated *in situ* in the spectral and kinetic determinations.

In the aqueous surfactant systems, the dependence of the equilibrium constant on pH was determined by use of an appropriate buffer. The pH was determined by a Radiometer PHM-26 instrument. Acetate, phosphate, and borate buffers were used in the ranges pH 4.5—6.0, 6.0—8.0, and 8.0—10.3, respectively.[23] Solubilities of hemin in Igepal CO-530–cyclohexane were determined spectrophotometrically by measuring the concentrations of saturated solutions. Excess of solid hemin was added to a series of cyclohexane solutions which contained various amounts of Igepal CO-530. After 3 d, aliquot portions of clear solution were taken, diluted, and the absorbances of the Soret band were measured against a blank. The concentrations of these saturated solutions were then calculated by use of calibration curves. The solubility of imidazole in cyclohexane was also determined spectrophotometrically by measuring the concentration of saturated solutions.

Absorption spectra and spectrophotometric equilibrium constants were obtained on a Cary 118-C spectrophotometer whose cell compartment was thermostatted at 25.0 ± 0.1 °C. Rate constants were determined using either Cary 118-C or Beckman Kintrac VII spectrophotometers and the Durrum model 110 stopped-flow system. The kinetic runs were usually maintained at 25.0 ± 0.1 °C using water circulation.

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RESULTS AND DISCUSSION

Solubilization and Absorption Spectra.—Hemin is only slightly soluble in cyclohexane; a 5.8×10^{-6} mol dm^{-3} solution showed no detectable absorbance. Addition of Igepal CO-530 resulted, however, in the development of a band centred at 395 nm. Stepwise increasing of the concentration of the surfactant caused slight bathochromatic shifts and increases in absorbance up to a maximum, after which there were no further changes (Figure 1). This behaviour is indicative of the solubilization of monomeric hemin by Igepal CO-530 aggregates in cyclohexane. A plot of hemin absorbances

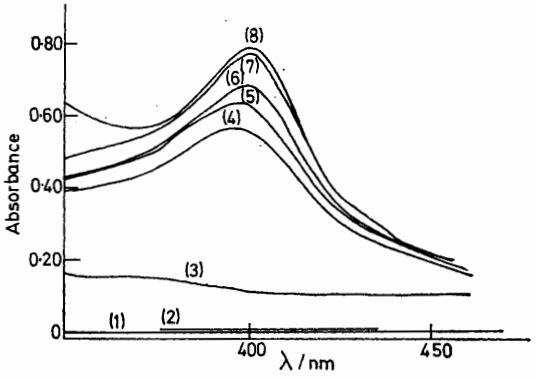

FIGURE 1 Absorption spectra of Soret band of 5.8×10^{-6} mol dm^{-3} hemin containing 1×10^{-4} mol dm^{-3} Na[OH] and 0.11 mol dm^{-3} water in (1) cyclohexane (no water), (2) 0.0136 mol dm^{-3} Igepal CO-530 in cyclohexane, (3) 0.034 mol dm^{-3} Igepal CO-530 in cyclohexane, (4) 0.068 mol dm^{-3} Igepal CO-530 in cyclohexane, (5) 0.136 mol dm^{-3} Igepal CO-530 in cyclohexane, (6) 0.340 mol dm^{-3} Igepal CO-530 in cyclohexane, (7) 0.68 mol dm^{-3} Igepal CO-530 in cyclohexane, and (8) pure Igepal CO-530. Recorded in 1.00 cm cells

against Igepal CO-530 concentrations at the absorption maxima yielded two straight lines (not shown) which intersect at a surfactant concentration corresponding to the operational critical micelle concentration. The spectral parameters of hemin in different solvent and surfactant systems are summarized in Table 1. Addition of up to 5% (v/v) of methanol did not alter the spectral parameters of hemin in cyclohexane in the presence of Igepal CO-530. In all the micellar systems employed, hemin obeyed Beer's law in the range 2×10^{-6}—4×10^{-5} mol dm^{-3}.

In the Igepal CO-530–cyclohexane system, addition of small amounts of water ($\leqslant$0.01 mol dm^{-3}) caused a very slow decay in the monomer absorbance, while larger additions (up to 4.50 mol dm^{-3}) caused a more rapid decay. These decay rates followed good pseudo-first-order kinetics. The decay is indicative of conversion of the monomeric form of hemin into its dimeric form. The added water presumably ' squeezes out ' the hemin from

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its reversed Igepal CO-530 micellar environment, where hemin is present in monomeric form, into the bulk cyclohexane environment, where hemin is present in dimeric form. Rate constants for this decay of monomeric hemin in cyclohexane at different concentrations

TABLE 1
Spectral parameters of hemin at 25.0 °C

Medium	Soret band $\lambda_{max.}$/nm	Soret band $10^{-4}\varepsilon$/dm^3 mol^{-1} cm^{-1}	Ref.
0.050 mol dm^{-3} htab in water [a]	400	6.4	19, *b*
0.50 mol dm^{-3} Na(ds) in water [a]	400	8.2	17, *b*
0.50 mol dm^{-3} Igepal CO-530 in cyclohexane [c]	402	6.5	*b*
0.50 mol dm^{-3} Igepal CO-530 in benzene [c]	402	8.1	*b*
0.50 mol dm^{-3} Igepal CO-530 in cyclohexane [d]	402	8.0	*b*
Ethanol–water (8.9 : 11.1 w/w) [a]	400	9.4	12
Methanol, 1.0 × 10^{-4} mol dm^{-3} Na(OMe)	398	11.6	15, *e*

[a] In buffered solutions at pH 6—10. See footnotes to Table 3 for details. [b] This work. [c] Containing 0.082 mol dm^{-3} methanol and (1—10) × 10^{-4} mol dm^{-3} Na(OMe). [d] Containing 0.144 mol dm^{-3} water and 1.0 × 10^{-4} mol dm^{-3} Na[OH]. [e] A. C. Maehly and A. Akeson, *Acta Chem. Scand.*, 1958, **12**, 1259.

of Igepal CO-530 and water ranged from 4.8×10^{-4} to 4.7×10^{-3} s^{-1}. Since these decay rates are relatively slow compared to rates of formation of the bis(imidazole) hemin product, they did not interfere in the determination of the rate or equilibrium constants.

Imidazole is highly soluble, of course, in water. Its solubility in benzene and dioxan is estimated from literature data to be 0.04 and 7.48 mol kg^{-1}, respectively.[24] The solubility of imidazole in neat cyclohexane was determined as $(5.0 \pm 0.8) \times 10^{-5}$ mol dm^{-3} by spectrophotometric measurement of the concentration of the supernatant in saturated solution. Addition of Igepal CO-530 to either benzene or cyclohexane greatly enhanced the imidazole solubility. For example, 1.5—2.0 mol dm^{-3} imidazole was readily solubilized in cyclohexane by 0.60 mol dm^{-3} Igepal CO-530. It is interesting that the solubility of hemin in cyclohexane increases linearly with increasing Igepal CO-530 concentration up to a point after which the solubility decreases. This break in the solubility curve depends on the amount of water present in the Igepal CO-530–cyclohexane system. At higher co-solvent concentrations, the break occurs at higher Igepal CO-530 concentrations. A plot of the water concentration against the concentration of Igepal CO-530 at which the hemin solubility begins to decrease gave a linear relation (not shown). The gradient of this plot is 5 ± 1 and can be considered as the minimum amount of water per surfactant required to keep the solubility dependence linear. It is possible to calculate the number of surfactant molecules surrounding each hemin from the relation [25] $[M] = (c_D - CMC)/N$, where [M] is the concentration of micelles, c_D is the stoicheiometric surfactant concentration, and N is the number of surfactants forming the aggregate. Since hemin is essentially insoluble in cyclohexane, assuming a 1 : 1 stoicheiometry between the surfactant aggregates and hemin, its solubility in a given surfactant solution actually represents the concentration of micelles.[15,25] From the gradient of the linear plot of hemin solubility against c_D — CMC, a value of $4\,000 \pm 800$ is calculated for N. This value can be compared with that of $2\,100 \pm 400$ determined for hemin in benzene in the presence of Igepal CO-530.[15]

Interaction of Imidazole with the Surfactant-solubilized Hemin Monomer.—Addition of increasing amounts of imidazole to the surfactant-solubilized hemin resulted in the development of a new absorbance at the expense of the band assigned to monomeric hemin. This new band is due to the bis(imidazole)hemin product. Table 2

TABLE 2
Spectral parameters of bis(imidazole)hemin at 25.0 °C

Medium	$\lambda_{max.}$/nm	$10^{-5}\varepsilon$/dm^3 mol^{-1} cm^{-1}
0.050 mol dm^{-3} htab in water [a]	416	1.10 [b] (0.95) [c]
0.50 mol dm^{-3} Na(ds) in water [a]	413	1.28 [b] (1.07) [c]
Ethanol–water (8.9 : 11.1 w/w) [a]	414	1.25 [d]
⩾0.20 mol dm^{-3} Igepal CO-530 in benzene or in cyclohexane [e]	414	1.06 [b] (0.90) [c]

[a] In buffered solutions at pH 6—10. See footnote to Table 3 for details. [b] Determined from limiting spectra. [c] Determined from a plot of equation (2). [d] Estimated from ref. 12. [e] Containing 0.082 mol dm^{-3} methanol and 1.0 × 10^{-4} mol dm^{-3} Na(OMe).

presents the spectral characteristics of bis(imidazole)-hemin and Figure 2 illustrates typical spectral changes for the interaction of imidazole (im) with hemin in the micellar environments. These data suggest the equilibrium formation of the bis(imidazole)hemin product as in (1). Good linear relations were obtained under

$$\left.\begin{array}{c}\text{hemin}(OH^-,H_2O)(S)\\ \text{or}\\ \text{hemin}(OMe^-,MeOH)(S)\end{array}\right\} + 2\text{im} \overset{K_{app.}}{\rightleftharpoons} \begin{array}{c}\text{hemin}(\text{im})_2(S) + OH^-/H_2O\\ \text{or}\\ \text{hemin}(\text{im})_2(S) + OMe^-/MeOH\end{array} \quad (1)$$

pseudo-first-order conditions (*i.e.* [im] ⩾ [hemin]) on plotting the left-hand side of equation (2) against

$$\frac{[\text{hemin}]_0}{A} = \frac{1}{\varepsilon} + \frac{1}{K_{app.}\varepsilon}\cdot\frac{1}{[\text{im}]^2} \quad (2)$$

$1/[\text{im}]^2$, where $[\text{hemin}]_0$ and [im] are the initial stoicheiometric concentrations of the reactants, A is the absorbance difference due to the bis(imidazole)hemin product at the appropriate wavelength maximum, $K_{app.}$ is the apparent equilibrium constant for its formation, and ε is the absorption coefficient. Values for the absorption coefficient for bis(imidazole)hemin determined from

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[25] J. H. Fendler, F. Nome, and H. C. Van Woert, *J. Amer. Chem. Soc.*, 1974, **96**, 6745.

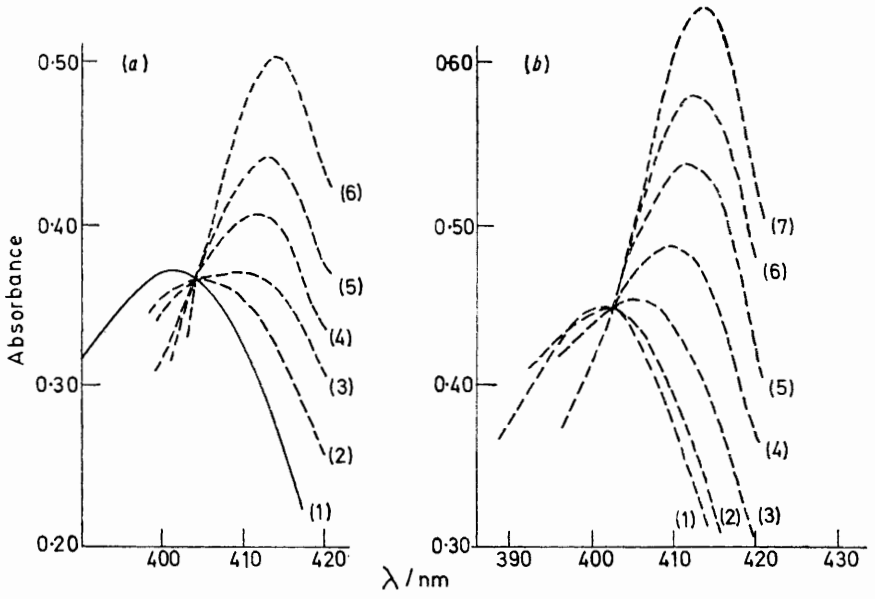

FIGURE 2 (*a*) Absorption spectra of 4.8×10^{-6} mol dm^{-3} hemin, containing 3.0×10^{-4} mol dm^{-3} Na(OMe) and 0.80 mol dm^{-3} Igepal CO-530 in benzene at 16.0 °C with (1) no added imidazole, (2) 8.58×10^{-4} mol dm^{-3} imidazole, (3) 1.23×10^{-3} mol dm^{-3} imidazole, (4) 1.72×10^{-3} mol dm^{-3} imidazole, (5) 2.58×10^{-3} mol dm^{-3} imidazole, and (6) excess of imidazole (0.01 mol dm^{-3}). Recorded in a 1.00 cm cell. (*b*) Absorption spectra of 5.86×10^{-6} mol dm^{-3} hemin in 0.05 mol dm^{-3} htab at pH 8.15 at 25.0 °C containing (1) no added imidazole, (2) 3.13×10^{-3} mol dm^{-3} imidazole, (3) 6.26×10^{-3} mol dm^{-3} imidazole, (4) 9.39×10^{-3} mol dm^{-3} imidazole, (5) 1.25×10^{-2} mol dm^{-3} imidazole, (6) 1.57×10^{-2} mol dm^{-3} imidazole, and (7) excess of imidazole (0.08 mol dm^{-3}). Recorded in a 1.00 cm cell

equation (2) are similar to those obtained from the limiting spectra (Table 2).

Alternatively, equation (3) may be used where A_{un}, A, and A_c are the absorbances due to hemin in the absence of imidazole, in the presence of different amounts of imidazole, and for the bis(imidazole)hemin product

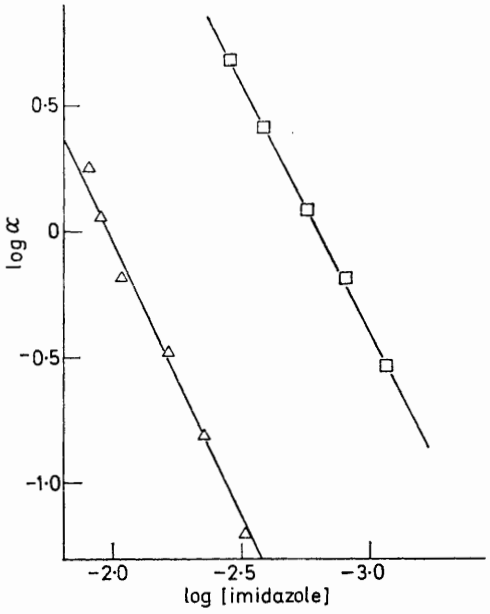

FIGURE 3 Plots of log α against log [imidazole] according to equation (3) for (△) 5.86×10^{-6} mol dm^{-3} hemin in 0.05 mol dm^{-3} htab in buffer (pH 8.15) at 25.0 °C containing various amounts of imidazole (gradient = 2.14), and (□) 4.80×10^{-6} mol dm^{-3} hemin in 0.80 mol dm^{-3} Igepal CO-530 in benzene at 16.0 °C containing 3.0×10^{-4} mol dm^{-3} Na(OMe) and various amounts of imidazole (gradient = 1.97)

formed with excess of imidazole, respectively. Plots of the logarithm of the left-hand side of equation (3) against

$$\frac{A_{un} - A}{A - A_c} = \frac{1}{K[\text{im}]^n} \quad (3)$$

the logarithm of the reciprocal of the imidazole concentration gave straight lines (Figure 3) with gradients of 2.0 ± 0.2 for all the systems studied. The value of 2 for the gradients substantiates the validity of equations (1) and (2) with respect to the involvement of two molecules of imidazole per molecule of hemin. In all the systems, the values of $K_{app.}$ calculated from equation (3) agreed well with those determined from (2).

The dependence of $K_{app.}$ on pH can be rationalized by two kinetically indistinguishable mechanisms [equations (4) and (5)]. If equation (4) is valid, then the apparent

$$\text{hemin}(OH^-,H_2O)(S) + \text{imH}^+ + \text{im} \overset{K'}{\rightleftharpoons} \text{hemin}(\text{im})_2 + 2H_2O \quad (4)$$

$$\text{hemin}(H_2O)_2(S) + 2\text{im} \overset{K''}{\rightleftharpoons} \text{hemin}(\text{im})_2 + 2H_2O \quad (5)$$

equilibrium constant should approximate to the right-hand side of equation (6). Alternatively, if equation (5)

$$K_{app.} = K'\left[\frac{K_H}{K_H + [H^+]}\right]\left[\frac{K_{imH}}{K_{imH} + [H^+]}\right]\left[\frac{[H^+]}{K_{imH} + [H^+]}\right] \quad (6)$$

governs the equilibrium formation of bis(imidazole)-hemin, $K_{app.}$ is expressed by equation (7) where $K_{app.}$ is the observed equilibrium constant at a particular pH,

K_{imH} and K_H are the dissociation constants for imidazole and hemin, respectively, and K' and K'' are the pH-independent equilibrium constants for equations (4) and (5).

$$K_{app.} = K''\left[\frac{K_{imH}}{K_{imH} + [H^+]}\right]^2\left[\frac{[H^+]}{K_H + [H^+]}\right] \quad (7)$$

Effects of Aqueous Micelles.—The apparent equilibrium constants for the binding of imidazole to hemin in aqueous 0.50 mol dm^{-3} sodium dodecyl sulphate and 0.05 mol dm^{-3} hexadecyltrimethylammonium bromide as functions of pH at 25 °C are in Table 3. Plots of $K_{app.}$

TABLE 3

Apparent equilibrium constants for the formation of bis(imidazole)hemin at 25.0 °C

0.05 mol dm^{-3} htab in water		0.50 mol dm^{-3} Na(ds) in water	
pH [a]	$10^{-4}K_{app.}$ [b]/dm^6 mol^{-3}	pH [a]	$10^{-6}K_{app.}$ [b]/dm^6 mol^{-2}
5.70 [c]	0.19 ± 0.02	6.00 [d]	0.080 ± 0.04
6.10 [d]	0.40 ± 0.05	6.20 [d]	0.135 ± 0.05
6.30 [d]	0.70 ± 0.10	6.85 [d]	0.790 ± 0.10
6.45 [d]	1.40 ± 0.08	7.00 [d]	1.40 ± 0.10
6.45 [e]	1.36 ± 0.10	7.20 [d]	2.20 ± 0.12
6.45 [f]	1.34 ± 0.09	7.30 [d]	3.74 ± 1.25
6.60 [d]	1.70 ± 0.08	7.45 [d]	4.50 ± 0.30
6.75 [d]	1.78 ± 0.08	7.80 [d]	4.20 ± 0.20
7.00 [d]	1.91 ± 0.14	8.00 [g]	3.60 ± 0.15
7.30 [d]	2.00 ± 0.08	8.20 [g]	4.20 ± 0.20
7.45 [d]	1.75 ± 0.12	8.40 [g]	3.50 ± 0.20
7.50 [d]	1.10 ± 0.10	9.00 [g]	1.70 ± 0.10
7.80 [d]	0.94 ± 0.05	9.50 [j]	0.60 ± 0.10
7.80 [e]	0.85 ± 0.08	10.35 [j]	0.030 ± 0.005
7.80 [f]	0.90 ± 0.08	10.95 [j]	0.006 ± 0.002
8.15 [g]	0.63 ± 0.09	11.30 [k]	0.002 ± 0.001
8.60 [g]	0.33 ± 0.09	11.60 [k]	0.0008 ± 0.0003
8.60 [h]	0.28 ± 0.05		
8.60 [i]	0.30 ± 0.06		
9.05 [g]	0.21 ± 0.08		
9.30 [j]	0.10 ± 0.04		
12.0 [l]	0.09 ± 0.05		

[a] Determined on a Radiometer pH M-26 instrument. [b] Determined by use of equation (2) or (3). [c] Buffer system employing 0.05 mol dm^{-3} potassium hydrogenphthalate which was adjusted to correct pH with standard Na[OH]. [d] Buffer system employing 0.05 mol dm^{-3} K[H_2PO_4] which was adjusted to appropriate pH with standard Na[OH]. [e] Buffer system employing 0.025 mol dm^{-3} K[H_2PO_4] which was adjusted to appropriate pH with standard Na[OH]. [f] Buffer system employing 0.10 mol dm^{-3} K[H_2PO_4] which was adjusted to appropriate pH with standard Na[OH]. [g] Buffer system employing 0.0125 mol dm^{-3} borax which was adjusted to appropriate pH with standard HNO_3. [h] Buffer system employing 0.050 mol dm^{-3} borax which was adjusted to appropriate pH with standard HNO_3. [i] Buffer system employing 0.0075 mol dm^{-3} borax which was adjusted to appropriate pH with standard HNO_3. [j] Buffer system employing 0.0125 mol dm^{-3} borax which was adjusted to appropriate pH with standard Na[OH]. [k] Adjusted to appropriate pH by use of standard Na[OH]. [l] 1.0 × 10^{-2} mol dm^{-3} Na[OH].

against pH (not shown) gave the typical bell-shaped curves. Using the available data for the dissociation constants of hemin and imidazole in the different environments (Table 4), both equations (6) and (7) were used to calculate the pH-independent equilibrium constants.

Regardless of the mechanisms followed, the order of stability of the bis(imidazole)hemin product is Na(ds) > EtOH–H_2O > htab. The same order of stability for bis(imidazole)hemin in aqueous micellar systems [Na(ds) > TX-100 (Triton X-100, a neutral micelle system) > htab] was recently reported from measurements carried out in the pH 8.45—11.4 region.[2a] A similar trend was reported for the reaction of imidazole with surfactant-solubilized heme *a* (the prosthetic group of cytochrome oxidase).[7] It is interesting to note that this trend is exactly the opposite to that observed for the interaction of cyanide ion with hemin or heme *a* in these aqueous micellar systems.[7,17-19] It appears that imidazole is more reactive than cyanide ion with iron porphyrin systems in anionic micellar solutions. The

TABLE 4

pK_a Values in different media

	Water	Ethanol–water (8.9 : 11.1 w/w)	5 × 10^{-2} mol dm^{-3} htab in water	5 × 10^{-1} mol dm^{-3} Na(ds) in water
Hemin	(7.50) [a]	6.63 [b]	6.10 [c]	5.50 [c]
Imidazole	7.06 [d]	6.74 [b]	7.13 [d]	7.94 [d]

[a] Ref. 1*e* and S. B. Brown, T. C. Dean, and P. Jones, *Biochem. J.*, 1970, **117**, 733; in dimeric form. [b] Ref. 12. [c] Ref. 19. [d] Ref. 25.

TABLE 5

pH-Independent equilibrium constants for the formation of bis(imidazole)hemin at 25.0 °C

Medium	K' [a] dm^6 mol^{-2}	K'' [b] dm^6 mol^{-2}
0.050 mol dm^{-3} htab in water	1.2 × 10^5	1.0 × 10^6
0.50 mol dm^{-3} Na(ds) in water	1.0 × 10^7	5.0 × 10^9
Ethanol–water (8.9 : 11.1 w/w) [c]	8.0 × 10^6	4.0 × 10^7

[a] Obtained by fitting experimental data to equation (6) (accurate to ±25%). [b] Obtained by fitting experimental data to equation (7) (accurate to ±25%). [c] Estimated from data presented in refs. 11 and 12.

reverse is the case, of course, in cationic micellar environments. Based on simple electrostatic considerations, it appears that cyanide and imidazolium ions are the reactive species. Additionally, the effects of micellar surfactants on the dissociation constants of both reactants (Table 4) need to be considered. As can be seen, hemin is less, but imidazolium ion is more, basic in micellar Na(ds) than in micellar htab or in aqueous ethanol. The behaviour of heme in micellar Na(ds) is analogous to that of iron complexes of substituted porphyrins where the stability increases as the basicity of the parent iron porphyrin decreases.[26] The greater stability of bis(imidazole)hemin in Na(ds) is probably due to the favourable electrostatic attraction between the reactive imidazolium ion and the solubilized hemin as well as to changes in the pK_a values of the reactants.[26] Based on published data on acidities of hemin,[26] the bis(imidazole)hemin product is expected to be more stable in micellar htab than in aqueous ethanol. In fact, the opposite is observed. This result is explicable in terms of unfavourable electrostatic interactions between the reactants which over-ride favourable micellar effects on hemin dissociation.

[26] V. N. Luzgina, E. I. Filippovich, G. N. Sakharova, and R. P. Eustigneeva, *Zhur. obshchei Khim.*, 1974, **44**, 2051.

The type of microenvironment in biological iron porphyrin reactive centres may be assessed by comparing their differential stability constants for cyanide and imidazole binding with those of simple hemes in various micellar systems.[7] Data for imidazole and cyanide binding to cytochrome oxidase (Fe^{3+}),[7] cytochrome *c* (Fe^{3+}),[5] and ferrihemochrome (hemin *c*) [27] are available. In these three systems the cyanide product has greater stability than the imidazole product, suggesting that the microenvironment about the iron reactive centre is in a 'cation-like' condition. It is also possible to utilize aqueous micelles as models for investigating the relations between the basicity of complex iron porphyrins and the stabilities of the products formed from them. This method would provide an alternative to the usual procedure of changing some secondary structure in the porphyrin.

The free energies of activation for the formation of the bis(imidazole)hemin complex, calculated from the pH-independent equilibrium constant [K', equations (4) and (6)], in aqueous 0.05 mol dm^{-3} htab and in 0.50 mol dm^{-3} Na(ds) are -28.9 ± 2.1 and -39.7 ± 2.1 kJ mol^{-1}, respectively. These values are somewhat smaller than that determined in 44% aqueous ethanol (-39.3 ± 1.7 kJ mol^{-1}).[11,12]

Effects of Reversed Micelles.—Apparent equilibrium constants for the formation of bis(imidazole)hemin in cyclohexane and in benzene in the presence of Igepal CO-530 are given in Table 6. The dependence of $K_{app.}$ on the surfactant concentration in cyclohexane can be seen by comparing the data in Table 6. It is seen that the stability of bis(imidazole)hemin increases with increasing concentrations of Igepal CO-530. This effect is the consequence of changes in the apparent dissociation constants of the reactants and in the acidities of surfactant-solubilized polar-solvent pools. The nature of these surfactant-entrapped polar solvents in bulk non-polar solvents is in many respects unique.[28] Indeed apparent dissociation constants for a variety of charged dyes localized in Igepal CO-530-solubilized water pools in benzene differ by up to seven orders of magnitude from those in bulk water.[29] Increasing the stoicheiometric concentrations of added nitric acid resulted in increases in $K_{app.}$ similar to those caused by increases in the surfactant concentrations. This implies that increasing amounts of Igepal CO-530 have the net effect of causing incremental increases of acidity in the co-solvent microenvironment about the reactive iron porphyrin centre. This curve (not shown) was approximately half the expected bell-shaped pH-dependence curve exhibited in aqueous micellar surfactants. At present it is impossible to obtain pH-independent equilibrium constants for the interaction of hemin with imidazole in surfactant-solubilized polar-solvent pools in organic solvents since values for meaningful pH and dissociation constants of the reactants are not available in this media.[28] Changing the co-solvent from methanol to water in the Igepal CO-530–cyclohexane system results in a 100-fold increase in $K_{app.}$ (Table 6). This again is accountable in terms of changes in the acidity of the hemin microenvironment and in the basicity of the reactants.

TABLE 6

Apparent equilibrium constants for the formation of bis(imidazole)hemin in organic solvents

Solvent	[Igepal CO-530] / mol dm^{-3}	$10^{-5}K_{app.}$ [a] / dm^6 mol^{-2}
Cyclohexane [b]	0.20	0.08 ± 0.005
	0.40	0.41 ± 0.06
	0.59	0.43 ± 0.07
	0.67	1.00 ± 0.11
	0.80	4.00 ± 0.27
	1.17	5.37 ± 0.25
	1.42	8.00 ± 0.26
Cyclohexane [c,d]	0.80	174.0 ± 15.0
Cyclohexane [c,e]	0.80	100.0 ± 10.0
Cyclohexane [c]	0.80	50.0 ± 4.0
Cyclohexane [c,f]	0.80	31.0 ± 3.0
Cyclohexane [g]	0.69	110.0 ± 2.0
Cyclohexane [h]	0.69	1.0 ± 0.5
Benzene [c,d]	0.80	77.6 ± 2.0
Benzene [c,e]	0.80	35.7 ± 2.0
Benzene [i]	0.80	13.5 ± 2.0

[a] Determined by use of equation (2) or (3) at 25.0 °C, unless stated otherwise. [b] Containing 0.082 mol dm^{-3} MeOH and 1.0×10^{-3} mol dm^{-3} Na(OMe). [c] Containing 0.082 mol dm^{-3} MeOH and 3.0×10^{-3} mol dm^{-3} Na(OMe). [d] At 9.5 °C. [e] At 16.0 °C. [f] At 32.0 °C. [g] Containing 0.144 mol dm^{-3} water and 3.0×10^{-4} mol dm^{-3} Na[OH]. [h] Containing 0.144 mol dm^{-3} MeOH and 3.0×10^{-4} mol dm^{-3} Na(OMe).

The stability of bis(imidazole)hemin is three times greater if cyclohexane rather than benzene is the bulk non-polar solvent. Presumably, the higher solubility of imidazole in benzene compared to that in cyclohexane affects a more favourable partitioning into the surfactant-solubilized polar-solvent pool localized in cyclohexane than that trapped in benzene. This effect is predominantly enthalpy governed. Values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ for the equilibrium formation of bis(imidazole)hemin in 0.80 mol dm^{-3} Igepal CO-530 [containing 0.080 mol dm^{-3} MeOH and 3.0×10^{-3} mol dm^{-3} Na(OMe)] in cyclohexane and benzene have been determined as -50.2 ± 8.4 kJ mol^{-1} and -39.7 ± 25 J K^{-1} mol^{-1}, and -69.0 ± 8.4 kJ mol^{-1} and -103.8 ± 29.3 J K^{-1} mol^{-1}, respectively.

In cyclohexane, in the presence of Igepal CO-530, the equilibrium attainment of bis(imidazole)hemin could be followed by stopped-flow spectroscopic methods. The kinetic data are in Table 7. The equilibrium formation of bis(imidazole)hemin occurs in two steps, (8) and (9).

$$\text{hemin}(OMe^-,MeOH)(S) + \text{im} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{hemin}(OMe^-,\text{im})(S) + \text{MeOH} \quad (8)$$

$$\text{hemin}(OMe^-,\text{im})(S) + \text{im} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{hemin}(\text{im})_2 + OMe^- \quad (9)$$

The kinetic rates are related to the apparent equilibrium constant by equation (10). The observed rate constants,

[27] N. Nanzyo and S. Sano, *J. Biol. Chem.*, 1968, **243**, 3431.
[28] J. H. Fendler, *Accounts Chem. Res.*, 1976, **9**, 153.
[29] F. Nome, S. A. Chang, and J. H. Fendler, *J. Colloid Interface Sci.*, 1976, in the press.

k_ψ, for product formation were determined from the gradients of linear plots of log $(A_\infty - A)$ due to product formation and log $(A - A_\infty)$ due to disappearance of original hemin against time. Assuming that the steady-state approximation holds (*i.e.* d[hemin(OMe$^-$,im)(S)]/dt = 0), these observed rate constants can be related to the imidazole concentration by equation (11).[12,15] At relatively high concentrations of imidazole, equation (11) simplifies to (12), while at low imidazole concentrations it approximates to (13). The data presented in Table 7 approximate well equation (11) and thus it is possible to estimate k_1 and k_{-2} at limiting values from equations (12) and (13). Alternatively, at high imidazole concentrations the data can be treated according to equation (14).[15,18,19] Equation (14) can be used to estimate (by calculation) the magnitude of k_{-1}/k_2 using the rate data obtained at high imidazole concentrations, and these obtained parameters are also included in Table 7. The two kinetically determined equilibrium constants agree fairly well with those determined from spectral data. The kinetic results show that the increase in $K_{app.}$ with increasing Igepal CO-530 is a result mainly of an increase in k_2/k_{-1}. Values of k_1 also increase with increasing surfactant concentrations, but this is offset by corresponding increases in k_{-2}.

$$K_{app.} = k_1k_2/k_{-1}k_{-2} \quad (10)$$

$$k_\psi = \frac{k_{-2}(1 + K_{app.}[im]^2)}{1 + (k_2/k_{-1})[im]} \quad (11)$$

$$k_\psi = k_1[im] \quad (12)$$

$$k_\psi = k_{-2} \quad (13)$$

$$\frac{[im]}{k_\psi} = \frac{k_{-1}}{k_1k_2} \cdot \frac{1}{[im]} + \frac{1}{k_1} \quad (14)$$

It is interesting to compare the rate data in the reversed micellar system (at 10^{-3} mol dm^{-3} added stoicheiometric base) with that reported for the 44.5% aqueous ethanol bulk-solvent system (in the range pH 9.72—10.3).[12] Except for the rate of the reverse of the second equilibrium step, k_{-2}, the values of K_{eq}, k_1, and k_2/k_{-1} are of the same order of magnitude. The pH dependence of K_{eq}, k_1, and k_2/k_{-1} would need to be known for accurate comparisons. However, the k_{-2} rates can be directly compared since these are pH independent.[12] The k_{-2} rates are 10—100-fold smaller in reversed micellar systems than in 44.5% aqueous ethanol (5—40 compared to 290—720 s^{-1}).[12] Apparently, the reversed micellar environment stabilizes the bis(imidazole) product to a greater extent than does the bulk 44.5% aqueous ethanol environment.

Similar results for k_{-2} (concerning dicyanohemin product) were noted on going from reversed micellar systems to methanol in the study of the reaction of cyanide with hemin.[15] The apparent equilibrium constant for the heme–imizadole complex in bulk methanol is *ca.* 80-fold less than that in the corresponding reversed micellar system. It is interesting to compare the rate data for the interaction of hemin with imidazole and cyanide ions in reversed micellar systems. Although of comparable overall stability, the dicyanohemin complex has smaller values for k_1 and k_{-2} than the bis(imidazole)-hemin complex. Thus Igepal CO-530 aggregates in non-polar solvents stabilize the dicyano-complex to a greater extent than the bis(imidazole) complex. The high solvent dependence of the Soret band for the dicyano-, but not for the bis(imidazole), complex is in accord with this observation.

TABLE 7

Interaction of hemin with imidazole in cyclohexane at 25.0 ± 0.1 °C

Conditions	10^3[imidazole] / mol dm^{-3}	$k\psi$ [a] / s^{-1}	k_1 [b] / dm^3 mol^{-1} s^{-1} [b]	k_2/k_{-1} [c]	k_{-2} [d] / s^{-1}	$10^5K_{app.}$ [e] / dm^6 mol^{-2}
0.80 mol dm^{-3} Igepal CO-530, 0.082 mol dm^{-3} MeOH, 1.0 × 10^{-3} mol dm^{-3} Na(OMe)	1.00	37.8	16 700 ± 3 000	310 ± 40	42 ± 3	1.25 ± 0.85
	2.00	43.0				
	4.00	47.8				
	7.00	78.8				
	9.25	109.1				
	12.50	163.0				
0.25 mol dm^{-3} Igepal CO-530, 0.82 mol dm^{-3} MeOH, 1.0 × 10^{-3} mol dm^{-3} Na(OMe)	5.00	3.76	3 400 ± 500	11.0 ± 4	5.0 ± 1	0.08 ± 0.01
	7.50	5.33				
	12.50	8.66				
	20.00	10.04				
	30.00	30.4				
	34.00	44.5				
	42.50	73.0				
	50.00	100.1				

[a] Determined from following the build-up of absorbance of the bis(imidazole) product at 414 nm. [b] Determined by using equation (12). [c] Determined by using equation (14). [d] Determined by using equation (13). [e] Determined by using equation (10).

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