Interactions and Reactions in Restricted Polar Media. Binding of Cyanide Ion to Hemin in Surfactant-solubilized Methanol in Benzene

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One molecule of monomeric hemin is solubilized by 2100 ± 400 molecules of uncharged surfactant Igepal CO-530 in benzene containing 0·2% (v/v) methanol. The substrate–surfactant binding constant in this system is (3·5 ± 0·5) × 10^4 l mol^{-1}. The environment of hemin in this restricted methanol pool is less polar than in bulk methanol. The interaction of cyanide ion with hemin, localized in the polar cavity of surfactant aggregates in benzene, has been treated in terms of a two-step process. Rate (k_1, k_{-1}, k_2, k_{-2}) and equilibrium (K) constants have been determined. Values for K, k_{-2}, k_2/k_{-1}, and k_1 in 0·70M Igepal CO-530 in benzene, containing 1·0% (v/v) methanol are 960-times greater, 200-fold smaller, 10-fold greater, and identical, respectively, to those in bulk methanol. The significance of these results are discussed and compared to those available in aqueous surfactant systems.

IMPORTANCE of hemoglobins in biochemical processes [1-4] has prompted the kinetic investigations of reactions involving hemin in aqueous and non-aqueous solutions.[5-10] Although these studies have provided a wealth of information, electrostatic, hydrophobic, and other microscopic medium effects of hemoproteins in their native environments could not be assessed. Dimerization of hemin as well as solvent co-ordination to the central iron atom introduced additional dissimilarities in the chemistry of hemin and hemoproteins. In order to minimize these problems Simplicio and his coworkers [5-7] have studied the interactions in and cyanide binding with aqueous micelle solubilized hemin. In these systems the hydrophobic interaction between the micelle and hemin apparently overcomes the hemin–hemin interactions. Kinetic and thermodynamic data could, therefore, be obtained for the binding of cyanide ion to hemin monomers.[5,6] Ligand substitution occurred through the penetration of cyanide ion across the ' membrane-like sheath imposed about the hemin at the micelle–water interface.' [5]

An alternative approach is to bring both the hemin monomer and cyanide ion, along with a limited amount of protic solvent, into a benzene solution by a suitable surfactant. We have reported that surfactants in nonpolar solvents form relatively small aggregates, termed as reversed micelles, which provide unique media for investigating interactions and reactions.[11] Possibilities of solubilizing controlled amounts of water or methanol in the polar cavities of these aggregates as well as binding substrates fairly rigidly accounts, among other factors, for the uniqueness of the system and allows its utilization as a model for interactions at enzyme-active sites and membrane processes. Indeed we have reported that rate constants for the mutarotation of 2,3,4,6-tetramethyl-α-D-glucose [12] and those for the decomposition of Meisenheimer complexes [13] in the polar cavities of

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[12] J. H. Fendler, E. J. Fendler, R. T. Medary, and V. A. Woods, *J. Amer. Chem. Soc.*, 1972, **94**, 7288.
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alkylammonium carboxylate surfactant aggregates in benzene are several orders of magnitude greater than those in pure benzene or in pure water. Aquation of the tris(oxalato)chromate(III) anion in the polar cavities of associated alkylammonium carboxylates proceeded six million-fold faster than that in pure water.[14,15] Even more significant is the observed specificity. Aquations of tris(oxalato)cobaltate or ethylenediaminebis(oxalato)chromate(III) are only modestly affected by surfactants in benzene.[16] It emerges that both the observed magnitude and specificity of catalyses in these systems significantly exceed those generally observed in aqueous micellar solutions.[17-20] It seemed to us worthwhile, therefore, to examine if relatively large molecules such as metal porphyrins could be solubilized in non-polar solvents by surfactants and if the rates of their reactions are affected in the restricted polar environments provided by this media. Availability of data on the interaction of hemin with cyanide ion in aqueous micellar systems [5-7] dictated this reaction to be the subject of our initial investigations.

EXPERIMENTAL

Hemin, ferriprotoporphyrin IX (Eastman), was used as received. Polyoxyethylene(6) nonylphenol, Igepal CO-530, was used as received from GAF Corporation. Sodium di(2-ethylhexyl) sulphosuccinate, Aerosol-OT (Aldrich), was dried prior to use. Reagent grade benzene was distilled from sodium and stored over Linde 5A molecular sieve. Stock solutions of hemin (generally 10^{-3}M) were usually made up under a stream of nitrogen in dry 0·05M-methanolic sodium methoxide and stored in the refrigerator. These solutions were stable for at least two weeks, as shown by spectrophotometry. Individual solutions for spectral and kinetic determinations were prepared by injecting appropriate volumes of the methanolic hemin stock solutions into benzene solutions of the surfactants. Final concentrations of hemin, methoxide ion, and methanol ranged between (5—8) × 10^{-6}M, (1—5) × 10^{-4}M, and 0·2—5·0% (v/v), respectively. Water concentrations, (9—10)10^{-4}M, in these solutions were carefully controlled and monitored by gas-liquid-partition chromatography using a Porapak Q column. Cyanide stock solutions were prepared by dissolving dry sodium cyanide (Baker Reagent grade) immediately prior to use in methanol and standardized by a spectroscopic method.[21] All other chemicals used were the best available reagent grade.

Isolation of Dicyanoferriprotoporphyrin Complex.—Hemin (0·100 g, 0·153 mmol) and sodium cyanide (0·186 g, 3·79 mmol) were shaken vigorously with water (1·0 ml) until coloured crystals appeared. After 15 min excess of acetone (5·0 ml) was added and the solvent was evaporated *in vacuo*. The solid was washed with benzene three times. The process was repeated subsequent to the addition of sodium cyanide. The isolated brownish crystals were dried *in vacuo*.

Solubilities of the isolated dicyanoporphyrin complex have been determined by measuring concentrations of saturated solutions at 25·0 °C spectrophotometrically. Excess of solid complex was added to a series of solutions containing different amounts of Igepal CO-530 in benzene–methanol 99 : 1% (v/v). These solutions were vigorously shaken for 2 h, allowed to stand overnight, and then centrifuged. Diluted aliquots were used to determine absorbances at 402 nm using appropriate blank solutions. Concentrations of the saturated solutions were then calculated using $\varepsilon_{402\ nm} = 8{\cdot}0 \times 10^4$ l mol^{-1} cm^{-1}.

Solubilities of sodium cyanide were determined by a modified spectroscopic method of Humphrey [22] on aliquots of saturated solutions in benzene both in the absence and presence of Igepal CO-530. A small volume (0·5 ml) of the cyanide solution was added to 2·0 ml of methanol. Addition of an excess of solid Hg_2Cl_2 to this solution resulted in a disproportionation reaction to form $Hg(CN)_2$. Subsequent addition of solid NaI results in the formation of $[HgI_4]^{2-}$, which absorbs at 300 nm ($\varepsilon = 1{\cdot}2 \times 10^4$ l mol^{-1} cm^{-1}). Calibration was obtained on standard cyanide solutions in Igepal CO-530 in benzene.

Spectrophotometric determinations were carried out using a Cary 118-C spectrophotometer whose cell compartment was thermostatted to 24·8 ± 0·1 °C. Kinetic data were obtained on a Cary 118-C, or a Beckman Kintrac VII spectrophotometer and on the Durrum model 110 stopped-flow T-jump system. Temperatures for the kinetic runs were maintained at 24·8 ± 0·1 °C by water circulation.

Critical micelle concentrations were determined by the dye method [23] using Bromophenol Blue.

RESULTS AND TREATMENT OF DATA

Hemin is completely insoluble in benzene. However, stable solutions of (6—8) × 10^{-6}M-hemin are obtained by injecting small volumes of methanolic stock solutions, in the presence of sodium methoxide, into benzene. Absorption spectra of these solutions [*i.e.* 6·2 × 10^{-5}M-hemin, 1·0 × 10^{-4}M-sodium methoxide in benzene in the presence of 0·02% (v/v) methanol] indicate hemin to be in aggregated form.[24] Successive addition of Igepal CO-530 to this solution results in an increase of absorption, parallel with a slight bathochromic shift in the Soret band, up to a maximum after which there is no further change (Figure 1). The molar absorptivity of hemin at 402 nm in benzene in the presence of 0·60M-Igepal CO-530, 8·1 × 10^4 l mol^{-1} cm^{-1}, is in good agreement with that reported [7] for hemin monomers in aqueous micellar sodium dodecyl sulphate (8·2 × 10^4 l mol^{-1} cm^{-1}), hexadecyltrimethylammonium bromide (7·0 × 10^4 l mol^{-1} cm^{-1}), and Triton X-100 (6·2 × 10^4M l mol^{-1} cm^{-1}). Furthermore the molar absorptivity of hemin in 0·60M-Igepal CO-530 in benzene was found to be unaffected by changes in the hemin [in the (2—20) × 10^{-6}M-range] or in the methanol [in the 0·02—4·0% (v/v) range] concentrations. Changes of the absorbances of hemin at 402 nm as functions of Igepal CO-530 concentrations are also

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illustrated in Figure 1. Drawing parallels to the two limbs of the obtained curve result in an intersection at $6\cdot5 \times 10^{-2}$M-Igepal CO-530. This value can be taken as the critical micelle concentration of the surfactant in benzene and it agrees well with that determined independently by the dye [$(4\cdot5—5\cdot5) \times 10^{-2}$M] and ^{1}H n.m.r.[25] [$(3\cdot5—5\cdot5) \times 10^{-2}$M] methods.

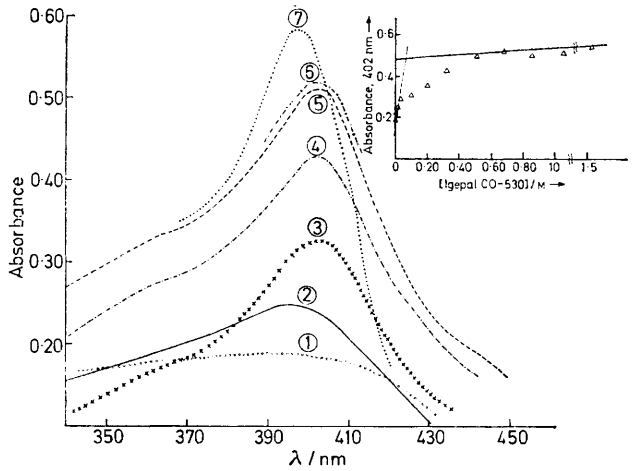

FIGURE 1 Absorption spectra of $6\cdot2 \times 10^{-6}$M-hemin, containing $1\cdot0 \times 10^{-4}$M-sodium methoxide and 0·02% (v/v) methanol in (1) benzene; (2) 0·02M-Igepal CO-530 in benzene; (3) 0·10M-Igepal CO-530 in benzene; (4) 0·32M-Igepal CO-530 in benzene; (5) 0·50M-Igepal CO-530 in benzene; (6) 1·2M-Igepal CO-530 in benzene; (7) neat methanol. Insert shows a plot of absorbance of $6\cdot2 \times 10^{-6}$M-hemin, containing $1\cdot0 \times 10^{-4}$M-sodium methoxide and 0·02% (v/v) methanol, in benzene at 402 nm *vs.* Igepal CO-530 concentration

Addition of Aerosol-OT to solutions of $6\cdot2 \times 10^{-6}$M-hemin in benzene, containing $1\cdot0 \times 10^{-4}$M sodium methoxide and 0·2% (v/v) methanol, resulted in the development of a new band at 407 nm with a shoulder at 378 nm. As the concentration of Aerosol-OT increased this shoulder developed into an absorption maxima. The development of the absorption maxima at 378 and 407 nm coincided with the aggregation of Aerosol-OT as established from a

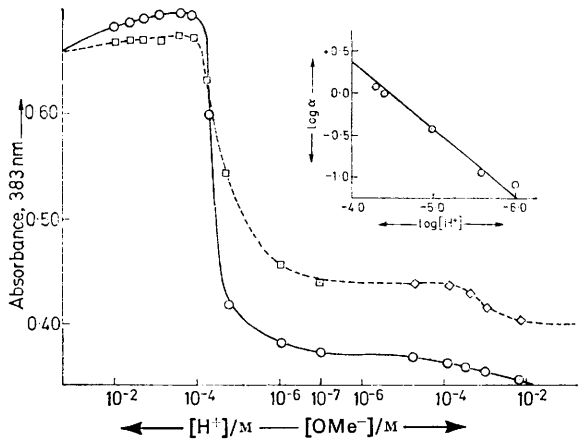

FIGURE 2 Changes of absorbance, at 383 nm, for $7\cdot0 \times 10^{-6}$M-hemin in benzene–methanol [99·6 : 0·4% (v/v)] containing 0·65M-Igepal CO-530, as functions of hydrogen-ion concentrations. Data on the dotted line were taken immediately after mixing while those on the solid line obtained 3 h subsequent to mixing. The logarithm of absorbance differences for the same data are plotted against the hydrogen-ion concentration in the insert

plot analogous to that illustrated in the insert of Figure 1. Formation of an additional peak at 378 nm is taken to indicate the replacement of solvent ligands of the hemin by Aerosol-OT molecules. Due to this complication the rest of the present investigations were limited to using Igepal CO-530.

Changing the stoicheiometric acidity of the Igepal CO-530 surfactant solubilized hemin in benzene, by the addition of appropriate amounts of sodium methoxide and nitric acid, results in significant absorbance changes. Such changes at 383 nm give the usual titration curve (Figure 2). On plotting absorbance differences logarithmically against the logarithm of stoicheiometric hydrogen ion concentration a straight line is obtained (see insert in Figure 2) from which an apparent pK_a value of $4\cdot4 \pm 0\cdot3$ is calculated for the equilibrium:

$$\text{Hemin(OMe,MeOH)}\cdot\text{(S)} + \text{H}^+ \rightleftharpoons \text{Hemin(MeOH)}_2^+\cdot\text{(S)} \quad (1)$$

The slope of this straight line, 0·8, substantiates a one to one interaction between the proton and the Igepal CO-530 solubilized hemin. Since subsequent investigations of the interaction of cyanide ion with hemin were carried out in the presence of sodium methoxide ($>8\cdot0 \times 10^{-5}$M) the unprotonated species is the reacting substrate. There is a slow change of absorbance over the entire acidity range (Figure 2). At base concentrations greater than 10^{-3}M it is due to dimer formation and at the lower base concentration it is accountable in terms of auto-oxidation of the vinyl side-chain of hemin.[26] In agreement with this suggestion is the observed decrease of the decay rate from $(4—8) \times 10^{-5}$ s^{-1} to $(8—12) \times 10^{-6}$ s^{-1} on careful exclusion of the oxygen by nitrogen purging. The time scale of subsequent kinetic investigations are considerably faster, this slow absorbance change can, therefore, be neglected.

Solubility of sodium cyanide in benzene is $2\cdot5 \times 10^{-4}$M. This value is in fair agreement with that extrapolated from known solubilities of NaCN in water and methanol using a correlation between solubilities and solvent polarity parameter, Z. Addition of Igepal CO-530 increases its solubility substantially (Table 1). A linear relationship is

TABLE 1

Solubilities of NaCN in Igepal CO-530 in benzene [a]

[Igepal CO-530]/M	Solubility of NaCN/M
0·000	$(2\cdot5 \pm 1) \times 10^{-4}$
0·030	$(3\cdot6 \pm 2) \times 10^{-4}$
0·060	$(8\cdot8 \pm 2) \times 10^{-4}$
0·090	$(9\cdot3 \pm 2) \times 10^{-4}$
0·126	$(13 \pm 5) \times 10^{-4}$
0·316	$(85 \pm 30) \times 10^{-4}$
0·633	$(59 \pm 30) \times 10^{-4}$
0·945	$(138 \pm 15) \times 10^{-4}$
1·260	$(186 \pm 10) \times 10^{-4}$
1·580	$(250 \pm 20) \times 10^{-4}$

[a] At ambient temperature; solubility of sodium cyanide in H_2O is 8·04M at 25·4 °C and that in MeOH is 1·24M at 25·1 °C.

obtained on plotting the left-hand side of equation (2) *vs.* $1/(c_D - c_{CMC})$: [19]

$$\frac{1-\alpha}{\alpha} = \frac{N}{\kappa} \cdot \frac{1}{(c_D - c_{CMC})} \quad (2)$$

where α is the solubility of NaCN in given Igepal CO-530 concentrations relative to that in benzene, κ is the binding

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constant between the surfactant and NaCN, c_D is the stoicheiometric Igepal CO-530 concentration and c_{CMC} is the critical micelle concentration. The slope of this line gives 1·42 for N/κ and assuming an aggregation number of 5 for Igepal CO-530 (ref. 25) a value of 3·5 ± 1 l mol^{-1} is estimated for κ.

Addition of increasing amounts of cyanide ion to the surfactant solubilized hemin, or that in pure methanol, results in increase of absorbances at 432·5 and 424 nm at the expense of absorptions at 402 nm and 398 nm for Igepal CO-530 and methanol, respectively. Figure 3 illustrates typical spectral changes for the interaction of cyanide ion with hemin in neat and Igepal CO-530 solubilized methanol. These data suggest the equilibrium formation of a new species:

$$\text{hemin(OMe,MeOH)}\cdot\text{(S)} + 2(\text{CN}^-) \overset{K}{\rightleftharpoons} \text{hemin(CN)}_2\cdot\text{(S)} + \text{OMe}^-/\text{MeOH} \quad (3)$$

Good relationships have been obtained under the pseudo-first-order conditions (*i.e.* $[\text{CN}^-] \gg [\text{hemin}]_0$) on plotting the left-hand side of equation (4) against $1/[\text{CN}^-]^2$

$$\frac{[\text{hemin}]}{A} = \frac{1}{\varepsilon} + \frac{1}{K\varepsilon}\frac{1}{[\text{CN}^-]^2} \quad (4)$$

where $[\text{hemin}]_0$ and $[\text{CN}^-]$ are the original stoicheiometric concentration of these reactants, A is the observed

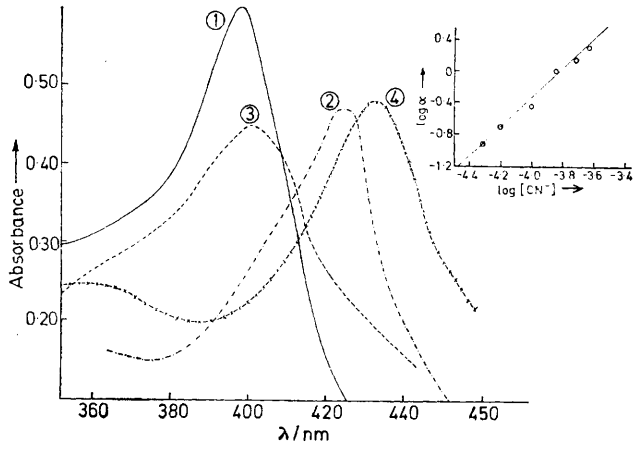

FIGURE 3 Absorption spectra of 6·0 × 10^{-6}M-hemin, containing 1·0 × 10^{-4}M-sodium methoxide: (1) in neat methanol; (2) in 2·8 × 10^{-3}M-NaCN in methanol; (3) in 0·70M-Igepal CO-530 in benzene; (4) in 0·70M-Igepal CO-530 and 7·2 × 10^{-4}M-NaCN in benzene. Data for the interaction of hemin with sodium cyanide in the 0·70M-Igepal CO-530 benzene system at 432·5 nm are plotted according to equation (5) in the insert

absorbance due to the dicyano-complex, at the appropriate wavelength, and K and ε are the equilibrium constant and molar extinction coefficient for hemin(CN)$_2$·(S). Values of 16·4 and 2·28 × 10^4 l mol^{-1} and 56 000 and 50 000 l mol^{-1} cm^{-1} have been obtained for K and ε in methanol and Igepal CO-530 in benzene, respectively.

Alternatively equation (5) can be used where A_{un}, A,

$$\frac{A_{un} - A}{A - A_c} = \frac{1}{K[\text{CN}^-]^n} \quad (5)$$

and A_c are absorbances due to hemin in the absence of cyanide ion, that in the presence of different amounts of cyanide ion, and that for the hemin(CN)$_2$·(S) complex, respectively. A plot of the logarithm of the left-hand side of equation (5) *vs.* the logarithm of the reciprocal cyanide ion concentration (illustrated for the Igepal CO-530–benzene system in the insert of Figure 3) gives straight lines with slopes of 2·2 ± 0·2 and 1·75 ± 0·05, for the interaction of hemin with cyanide ion in benzene in the presence of Igepal CO-530 and in neat methanol, respectively. These values substantiate the validity of equation (3) with respect to the involvement of two

TABLE 2

Interaction of hemin with cyanide ion

10^4[CN$^-$]/M	k_ψ/s^{-1}	Abs.
In methanol [a]		
5·00	0·0870	0·018
10·00	0·0620	0·080
20·00	0·110	0·179
40·00	0·240	0·279
50·00	0·380	0·310
65·00	0·560	0·315
97·50	1·24 (0·98) [b]	0·321
150·0	2·42 (2·18) [b]	
195·0	4·00	0·317
308·0	6·82 (6·56) [b]	
600·0	18·28 (18·28) [b]	0·321
0·70M-Igepal CO-530 in benzene [c]		
0·479 [d]		0·040
0·980 [d]	0·00184	0·098
1·467 [d]	0·00164	0·175
1·904 [d]	0·00596	0·230
2·376 [d]	0·0095	0·260
7·128 [d]	0·036	0·310
15·0 [e]	0·117 (0·122) [f]	0·310
30·0 [e]	0·276	0·315
55·0	0·660	
60·0 [e]	0·874 (0·904) [f]	0·325
100·0	1·70	
120·0 [e]	2·87 (2·73) [f]	0·325
0·70 M-Igepal CO-530 in benzene [g]		
31·0	0·341	
40·0	0·262	
50·0	0·585	
100	1·66	
160	2·90	
310	6·30	
0·70M-Igepal CO-530 in benzene [h]		
0·76	0·00155	
1·52	0·00690	
2·28	0·00920	
3·04	0·0144	
3·80	0·0253	
5·00	0·0416	
1·35M-Igepal CO-530 in benzene [g]		
1·95	0·00425	
3·90	0·00666	
5·85	0·0161	
7·80	0·0198	
9·75	0·0269	
11·7	0·0423	
21·0	0·140	
45·0	0·345	
90·0	0·715	
120	1·08	
141	1·35	
280	3·15	

[a] In pure methanol, containing 1·0 × 10^{-4}M-NaOMe. [Hemin] = 6·5 × 10^{-6}M at 24·0 °C. Followed at 424 nm (build-up of the dicyano-complex) unless otherwise stated. [b] Followed at 398 nm (decay of parent). [c] Solution contains 1% methanol (v/v), and (8·6—20) × 10^{-5}M-NaOMe. [Hemin] = 6·7 × 10^{-6}M. Absorbances determined at 432·5 nm (build-up of dicyano-complex) unless otherwise stated. [d] 24·6 °C. [e] 24·8 °C. [f] Followed decay of parent at 402 nm. [g] 5% MeOH, 1 × 10^{-4}M-OMe. [Hemin] = 5·0 × 10^{-5}M at 25·0 °C. [h] Solution contains 0·15% methanol (v/v), 1·0 × 10^{-4}M-NaOMe and 5·0 × 10^{-7}M-hemin. Followed at 433·5 nm at 24·8 °C.

molecules of cyanide ion per one molecule of hemin. Values of K (15 and $2{\cdot}4 \times 10^4$ l mol^{-1}) calculated from equation (5) agree, of course, with those obtained using equation (4) ($16{\cdot}4$ and $2{\cdot}3 \times 10^4$ l mol^{-1}).

Depending on the concentration of cyanide ion, the attainment of equilibrium (3) could be followed either by conventional or stopped-flow spectroscopic methods. The data are given in Table 2. Equilibrium formation of the hemin$(CN)_2$·(S) complex occur in two steps:

$$\text{hemin(OMe,MeOH)·(S)} + CN^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{hemin(OMe,CN)·(S)} + \text{MeOH} \quad (6)$$

$$\text{hemin(OMe,CN)·(S)} + CN^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{hemin}(CN)_2\text{·(S)} + {}^-\text{OMe} \quad (7)$$

and equations (6) and (7) are related to the association constant [equation (3)] by relation (8). Assuming that the

$$K = \frac{k_1 k_2}{k_{-1} k_{-2}} \quad (8)$$

steady-state approximation holds (*i.e.* d[hemin(OMe,CN)·(S)]/dt = 0) the observed rate constant for the decay of the absorption due to the disappearance of hemin(OMe,MeOH)·(S) or that for the increase of absorbance due to the formation of hemin$(CN)_2$·(S), k_ψ, is given by relation (9):

$$k_\psi = \frac{k_{-2}(1 + K[CN^-]^2)}{1 + (k_2/k_{-1})\,[CN^-]} \quad (9)$$

Equation (9) at high cyanide concentration simplifies to:

$$k_\psi = k_1[CN^-] \quad (10)$$

while at low cyanide ion concentration it approximates to:

$$k_\psi = k_{-2} \quad (11)$$

The data obtained (Table 2) approximate well equation (9) and taking limiting slopes at high and low cyanide ion concentration (as illustrated in Figure 4) values for k_1 and k_{-2} are obtained and given in Table 3. Alternatively the data can be treated according to equation (12).[7] Good

$$\frac{[CN^-]}{k_\psi} = \frac{k_{-1}[\text{OMe}]}{k_1 k_2 [CN^-]} + \frac{1}{k_1} \quad (12)$$

linear relationships have been obtained on plotting the left-hand side of equation (12) *vs.* 1/[CN^-] (see insert in Figure 4), from the intercepts and slopes of which k_1 and k_{-1}/k_1k_2 are obtained. These values are also given in Table 3.

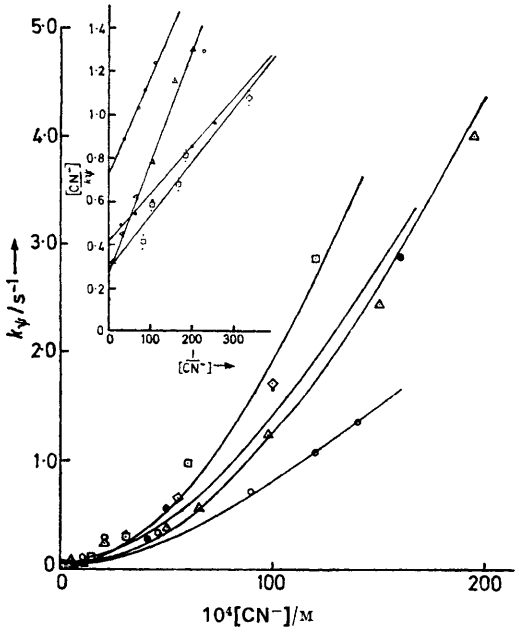

FIGURE 4 Plot of the observed rate constants, k_ψ, for the interaction of hemin with cyanide ion *vs.* stoicheiometric cyanide ion concentration. Data are plotted according to equation (12) in the insert. (△) In neat methanol; (⊡) in benzene in the presence of 0·70M-Igepal CO-530 and 1·0% (v/v) methanol; (●) in benzene in the presence of 0·70M-Igepal CO-530 and 5·0% (v/v) methanol; (○) in benzene in the presence of 1·35M-Igepal CO-530 and 5·0% (v/v) methanol

TABLE 3

Kinetic and thermodynamic parameters for the interaction of hemin with cyanide ion

Conditions	k_1/ l mol^{-1} s^{-1} [a]	k_1/ l mol^{-1} s^{-1} [b]	k_2/k_{-1} [OMe] [b]	k_{-2}/s^{-1} [c]	k_{-2}/s^{-1} [d]	K/l mol^{-1} [e]	K/l mol^{-1} [f]
MeOH, $1{\cdot}0 \times 10^{-4}$ MeONa, 24 °C	365 ± 30	385 ± 10	(3·6 ± 0·6)10^{-3}	(6 ± 2)10^{-2}	(4 ± 0·5)10^{-2}	26·0 ± 1·0	16·4 ± 0·5
0·70M-Igepal CO-530 in benzene, 1·0% MeOH (v/v), $1{\cdot}0 \times 10^{-4}$M-MeONa, 24·8 °C	388 ± 20	350 ± 50	(3·6 ± 0·5)10^{-2}	(3 ± 1)10^{-4}	(7·1 ± 0·5)10^{-4}	(2·5 ± 0·8)10^4	(2·3 ± 0·5)10
0·70M-Igepal CO-530 in benzene, 0·15% MeOH (v/v), $1{\cdot}0 \times 10^{-4}$M-MeONa, 24·8 °C		170 ± 30	(3·8 ± 0·3)10^{-2}	(8 ± 3)10^{-4}	(1·2 ± 0·3)10^{-3}	(1·2 ± 0·5)10^4	
0·70M-Igepal CO-530 in benzene, 5% MeOH (v/v), $1{\cdot}0 \times 10^{-4}$M-MeONa, 25·0 °C	210 ± 20	250 ± 30	(1·9 ± 0·7)10^{-2}		(2·0 ± 0·5)10^{-3}	(2·4 ± 0·3)10^3	
1·35M-Igepal CO-530 in benzene, 5·0% MeOH (v/v), $1{\cdot}0 \times 10^{-4}$M-MeONa, 25·0 °C	80 ± 5	105 ± 20	(5·5 ± 0·4)10^{-2}	(2·1 ± 0·4)10^{-3}	(2·8 ± 0·6)10^{-3}	(2·0 ± 0·2)10^3	
4% CTAB in H_2O [g]		11200 ± 2000	(2·56 ± 0·2)10^{-2}			(8·8 ± 1·8)10^4	(5·9 ± 1·2)10^4
2% NaLS in H_2O [g]		3400 ± 185	2·75 × 10^{-4}		5·7 × 10^{-3}	16·4	14·4 ± 4
3% Triton X-100 [g]		510 ± 60	1·25 × 10^{-3}			2·16 × 10^2	1·72 × 10^2

[a] Using equation (10). [b] Using equation (12). [c] Using equation (11). [d] From the decay of isolated hemin$(CN)_2$·(S). [e] Using equation (8) and multiplying by [OMe]. [f] Using equation (4) and multiplying by [OMe]. [g] Taken from ref. 7.

Rate constants for the decomposition of hemin$(CN)_2$·(S), k_{-2}, were obtained independently by dissolving the isolated complex (see Experimental section) in the appropriate solvent system and following either the decay of absorbance due to this species or, alternatively, the appearance of the parent peak due to hemin(OMe,MeOH)·(S) as a function of time. Values of k_{-2} obtained by this method agree well with those determined by using equation (11) (Table 3). Values for k_{-2}, using the isolated complex, have also been obtained in benzene at different concentrations of surfactant and methanol. Table 4 summarizes the data. At constant methanol concentration increasing the surfactant concentration results in an increase in k_{-2}. Similarly, at constant surfactant concentration the rate is enhanced by increasing methanol concentration. This latter dependency allows the calculation of the second-order rate constant for the decomposition of hemin$(CN)_2$·(S) with respect to methanol.

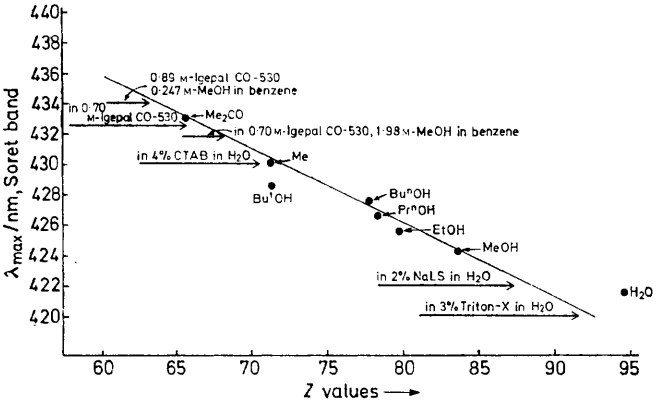

FIGURE 5 Correlation between the absorption maxima of the Soret band of the isolated hemin$(CN)_2$ complex and solvent polarity parameter Z

The obtained value in 0·89M-Igepal CO-530 in benzene, $1{\cdot}02 \times 10^{-3}$ l mol^{-1} s^{-1}, agrees well with $1{\cdot}6 \times 10^{-3}$ l mol^{-1} s^{-1} calculated for the same reaction in neat methanol (by dividing 24·7). The absorption maximum of the Soret band of the isolated dicyanohemin complex is highly solvent sensitive. A good relationship is, in fact, obtained on plotting its absorption maxima against the microscopic solvent polarity parameter, Z (Figure 5). Such correlation allows the estimation of the microscopic environment of the hemin complex in the surfactant-solubilized methanol (Figure 5).

DISCUSSION

Micelles and surfactant aggregates interrupt hemin–hemin interactions both in aqueous [5-7] and in benzene solutions. It is instructive to compare the magnitude and site of solubilization of hemin as well as its reactivity in aqueous and non-aqueous surfactant systems. In aqueous anionic and uncharged micelles, hemin is predominantly solubilized at the micelle–water interface.[5] Indeed, deep substrate penetration into the micellar core in aqueous solutions rarely occurs.[18] Conversely, in benzene hemin is predominantly localized in the methanol pool which is surrounded by surfactant molecules. Positions of the absorption maxima of the dicyanohemin complex in the different surfactant systems substantiate this postulate. Using the linear correlation obtained between the absorption maxima of the Soret band of hemin$(CN)_2$·(S) in solvents of different polarities *vs.* the solvent polarity parameter Z (Figure 5), the apparent environment of hemin$(CN)_2$ complex in aqueous sodium dodecyl sulphate and Triton-X [7] is water like (*i.e.* the extrapolated Z values are 92·5 and 88·5). Cationic micellar hexadecyltrimethylammonium bromide intercalated hemin is in an apparently less-polar environment than it is in the other aqueous surfactants. Manifestation of the most pronounced micellar effects in aqueous cationic surfactants [7] can, therefore, be rationalized in terms of deeper substrate penetration. The extrapolated Z value for hemin$(CN)_2$·(S) in benzene in the Igepal CO-530 system depends on the concentration (*i.e.* the size) of the methanol pool. The apparent polarity of hemin solubilized in benzene by 0·89M-Igepal CO-530 in the presence of 0·24M-MeOH is less than acetone. Increasing the concentration of solubilized methanol or decreasing the concentration of Igepal CO-530 result in an increase of the microscopic polarity of the hemin molecule (Figure 5). Changing the concentration of the polar solvent can, therefore, bring about changes in the effective polarities of hemin.

Information on the number of Igepal CO-530 molecules utilized for the solubilization of one hemin molecule have been obtained from solubility measurements (see Experimental section). The concentration of micelles, [M], is given [19] by:

$$[\mathrm{M}] = \frac{c_{\mathrm{D}} - c_{\mathrm{CMC}}}{N} \qquad (13)$$

Since hemin is completely insoluble in dry benzene, assuming a 1 : 1 interaction between hemin and the surfactant, its solubility in given surfactant solutions represents the concentration of micelles (*i.e.* hemin is being used to titrate the micelles). A good straight line has been obtained on plotting hemin solubilities, that is micelle concentrations, against $(c_{\mathrm{D}} - c_{\mathrm{CMC}})$ [equation (13)]. From the slope of this line a value of 2100 ± 400 is calculated for N. Although the assumptions involved in this calculation may not entirely be valid, the number of surfactant molecules needed to solubilize a hemin molecule is considerably greater than the range of aggregation numbers quoted for reverse micelles in non-polar solvents.[27] Evidently such a large molecule as hemin is solubilized in non-polar solvents by being wrapped around surfactant molecules of some thickness, forming grossly enlarged aggregates. We have encountered similarly large dodecyl ammonium propionate aggregates in the solubilization of vitamin B_{12a}.[28] Since no information is available on the shape of surfactant aggregates containing large porphyrin molecules in benzene, we are somewhat reluctant to call them ' reversed micelles ' in the accepted sense of terminology.[27] Lack of data on the size of aqueous micelles in the

[27] F. M. Fowkes in ' Solvent Properties of Surfactant Solutions,' ed. K. Shinoda, Marcel Dekker, New York, 1967, p. 65.

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

presence of hemin does not allow comparisons between the extent aqueous and non-aqueous aggregates enlarge upon solubilizing hemin.

From the decomposition of hemin$(CN)_2$·(S) in benzene solutions containing different amounts of Igepal CO-530

TABLE 4

Rate constants for the decomposition of isolated hemin$(CN)_2$·(S) complex in benzene at 24·8 °C

[Igepal CO-530]/M	[MeOH]/M	10^3k_{-2}/s^{-1} [a]	10^3k_{-2}/s^{-1} [b]
0·890	0·0	0·81	
0·890	0·247	1·03	
0·890	0·494	1·30	1·31
0·890	0·988	1·80	
0·890	1·98	2·83	
0·175	0·247 [c]	0·28	0·32
0·350	0·247 [c]	0·40	0·42
0·700	0·247 [c]	0·72	0·74
1·43	0·247 [c]	0·84	0·85
0·70	1·235 [c]	2·10	
1·35	1·235 [c]	2·80	

[a] Obtained by following the decay of absorbance due to hemin$(CN)_2$·(S). [b] Obtained by following the increase of absorbance due to hemin(OMe,MeOH)·(S). [c] In the presence of 1×10^{-4}M-MeONa.

(Table 4) the magnitude of substrate–surfactant binding constant, κ, can be estimated by using equation (14):[19]

$$\frac{1}{k^{\psi}_{-2}} = \frac{1}{k^{m}_{-2}} + \frac{1}{k^{m}_{-2}}\left(\frac{1}{c_D - c_{CMC}}\right)\frac{N}{\kappa} \qquad (14)$$

where k^{ψ}_{-2} and k^{m}_{-2} are the observed first-order rate constants at given Igepal CO-530 concentrations and in the micellar phase. A plot of the left-hand side of equation (14) against $1/(c_D - c_{CMC})$ gives a good straight line from which a value of 0·06 is calculated for N/κ. Taking a value of 2100 ± 400 for N, as obtained from solubility measurements [equation (14)], κ is estimated to be $(3{\cdot}5 \pm 0{\cdot}5) \times 10^4$ l mol^{-1}. Hemin is apparently appreciably bound to Igepal CO-530 in the benzene–methanol $99{\cdot}8 \pm 0{\cdot}2$ (v/v) solvent system.

Rate and equilibrium constants for the formation of hemin$(CN)_2$·(S) complex are strongly medium dependent (Table 3). The observed differences among the differently charged aqueous micelles have been rationalized primarily on electrostatic grounds.[7] Dimer formation precludes, of course, the availability of data in water. Effects of Igepal CO-530 in benzene containing small volumes of methanol can be related, however, to that in bulk methanol. It is seen that equilibrium constants in benzene in the presence of 0·70M-Igepal CO-530 are up to 960-fold greater than that in methanol (Table 3). The magnitude of this enhancement decreases with increasing volumes of methanol in the Igepal CO-530 system. Increasing the size of the methanol pool will not only decrease the beneficial effect of substrate–surfactant interactions but will likely alter the effective activity of methanol. Analogous and even more pronounced effects have been observed in ligand exchange reactions at vitamin B_{12a} in surfactant solubilized water in benzene.[28]

Effects of surfactant aggregates on the equilibrium constant is a composite of the individual rate constants [see equation (8)]. Of these, values for k_1, k_{-2}, and k_2/k_{-1} are available (Table 3). Effects on the rate constant for the decomposition of hemin$(CN)_2$·(S), k_{-2}, are the most straightforward to interpret since it represents a unimolecular process. In aqueous micellar NaLS the value for k_{-2} is identical, within experimental errors, to that in methanol. Conversely, values for k_{-2} are factors of up to 200-fold smaller in surfactant-solubilized methanol than in methanol (Table 3). This rate retardation is likely to be the consequence of enhanced ground-state stability of hemin$(CN)_2$·(S) in the restricted methanol pool. Surprisingly a 10-fold reduction of the methanol pool (to 0·15% v/v methanol) does not affect further the inhibition of k_{-2} (Table 3). Rate-constant ratios for k_2/k_{-1} are enhanced up to a factor of 10 in the benzene-solubilized methanol with respect to bulk methanol. Enhancements of the rate constants for cyanide addition to hemin, k_1, in aqueous micellar systems (Table 3) have been largely rationalized on electrostatic considerations.[7] Absence of surfactant effects in benzene is necessarily a composite of hemin stabilization, and the meagre surfactant cyanide ion association.

CONCLUSION

The present results demonstrate the utility of surfactants to solubilize such a large molecule as hemin in pools of protic solvents in bulk benzene. In many respects this system bears analogy to hydrophilic pockets in native proteins and membranes. The observed structural and reactivity differences of hemin in the bulk solvent and in the cavities of surfactant aggregates question the validity of extrapolating information from dilute aqueous solutions to complex biochemical systems in a straightforward manner. We are extending these investigations to other reactions involving hemins as well as other porphyrins.

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