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Electrochemical behaviour of (protoporphyrinato IX)iron(III) encapsulated in aqueous surfactant micelles

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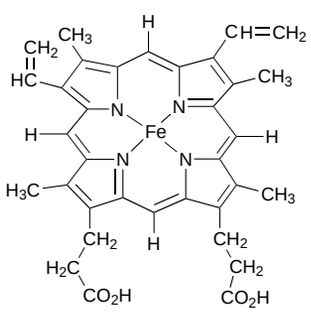
The electrochemical behaviour of iron protoporphyrinate IX (hemin) [(3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoato)iron(III)] in aqueous sodium dodecyl sulfate (sds), hexadecyltrimethylammonium bromide or Triton X-100 surfactant micellar solution was investigated by cyclic voltammetry (CV) and Osteryoung square-wave voltammetry (OSWV) techniques. The dependence of the midpoint potential on the concentration and on the nature of the surfactant shows that the potentials shift anodically with respect to water or aqueous ethanol. The midpoint potentials of hemin at pH 7.0 *vs.* normal hydrogen electrode are −52 mV in $NMe_3(C_{16}H_{33})Br$, −112 mV in Triton X-100, −152 mV in sds and −190 mV in ethanol–water (1 : 1, v/v) solutions. Thus the hydrophobic effect of the micelle gives a positive shift of the midpoint potential. The maximum positive shift in surfactants (with respect to water) of *ca.* +120 mV was found in the micelles. The trend in the anodic shift is EtOH–water < sds < Triton X-100 < $NMe_3(C_{16}H_{33})Br$. The diffusion coefficients of the hemin complex in the micelles are an order of magnitude smaller than that of monomeric hemin in aqueous ethanolic media. The rates of heterogeneous electron transfer at the glassy carbon electrode were found to be smaller in the micelles as compared to those in aqueous ethanolic media. The midpoint potential of hemin monomers encapsulated in aqueous surfactant micelles shows a pH dependence with $\Delta E/\Delta pH$ *ca.* −59 mV indicating that electron transfer at the iron site is influenced by the uptake of protons at the axial ligand.

The heme proteins are widely distributed in cellular systems, catalysing a variety of biological processes, primarily redox reactions essential for respiration and production of metabolic energy.[1–3] The heme in proteins exhibits a large positive redox potential[4,5] relative to model complexes in aqueous solutions [*e.g.* +47 mV in myoglobin and +260 mV in cytochrome c as compared to *ca.* −200 mV *vs.* normal hydrogen electrode (NHE) in model heme complexes]. These large values were attributed to the hydrophobic environment of apoproteins around the heme.[4,5] It was shown[4,5] that a very low value of the effective relative permittivity of the local heme environment in the protein pocket can lead to a large positive redox potential. However, there is a dearth of experimental evidence to show that the hydrophobic microenvironment around a heme in an essentially aqueous medium can indeed lead to a large positive redox potential.

An area of current research interest is to obtain a more precise physical and chemical description of redox-linked translocation of protons in biological membranes.[4] There are several heme proteins where an equilibrium of an ionisable functional group controls the redox potential of the metal.[4]

An attractive model for study of the electron transfer in hemoproteins is (protoporphyrinato IX)iron(III) [(3,7,12,17-tetramethyl-8,13-divinylporphyrin-2,18-dipropanoato)iron(III)], commonly known as hemin, which is present in the prosthetic group of cytochromes b and peroxidases.[1,2] Unfortunately, the natural porphyrins are sparingly soluble in water at low pH and undergo extensive aggregation[6] at alkaline pH. However hemin is soluble in water over a wide range of pH in the presence of aqueous surfactant micelles.[7] In aqueous surfactants it exists as a monomer[7–10] with a radial alignment[8] of the porphyrin within the micelles. The nature of iron-(III) and -(II) porphyrins encapsulated in micelles has been investigated by various techniques. At low pH (*ca.* 2.6) the species in solution is the diaqua ion[7,11,12] $[FeL(H_2O)_2]^+$, while at high pH (*ca.* 7.0) the aquahydroxo complex, $[FeL(OH)(H_2O)]$, is predominant in aqueous solution[7–12] (H_2L = protoporphyrin IX). The immediate microenvironment of hemes in aqueous surfactants is predominantly non-polar or hydrophobic[7] and the surface charges of the micelles have considerable influence on the reactivity of hemin.[7]



Electrochemistry of hemes in aqueous solutions is complicated by solubility restrictions and aggregation behaviour.[13,14] The hemins undergo a reversible reduction and the overall reaction is a two-electron process, iron(III) dimer ⟶ iron(II) monomer.[15] The reduction product of the dimer in alkaline solution is strongly adsorbed at the electrode.[15] When the ionic strength of the medium was 0.1 M and the concentration of hemin *ca.* 1 mM, a diffusion-controlled process was detected[16] in the range pH 7–13. The midpoint potential in water is pH dependent and the reduction involves two electrons and two protons (or two OH ions) with a pK_a^{III} at 7.5 and pK_a^{II} more than 12.5 for the iron-(II) and -(III) complexes, respectively.[14]

Electrochemical study of metal complexes in aqueous surfactant micelles is an area of current research interest.[17–20] Hence it would be of interest to study monomeric hemes in aqueous surfactant solutions over a wide range of pH by electrochemical techniques. In this paper we report the midpoint potential of monomeric hemin encapsulated in three different surfactant micelles, measured by cyclic voltammetry (CV) and Osteryoung square-wave voltammetry (OSWV). The surfactants used here are anionic sodium dodecyl sulfate (sds), neutral Triton X-100 and cationic hexadecyltrimethylammonium bromide. The pH dependence of the midpoint potentials of

Table 1 Electrochemical results for hemin (1.0 mM) in aqueous media (glassy-carbon electrode, reference electrode Ag–AgCl, supporting electrolyte 0.1 M $NaNO_3$; pH 8.0; buffer 0.05 M Tris–HCl; scan rate 100 mV s^{-1}

	$E_{\frac{1}{2}}$/V					
System	CV	OSWV	ΔE_p/mV	i_{pc}/i_{pa}	$10^7 D_0$/cm^2 s^{-1}	$10^3 K_s$/cm s^{-1}
4% $NMe_3(C_{16}H_{33})Br$	−0.312	−0.304	77	1.07	1.5	1.8
4% Triton X-100	−0.370	−0.376	70	1.33	3.6	1.1
4% sds	−0.410	−0.404	73	1.00	5.3	2.2
Ethanol–water (1 : 1 v/v)	−0.444	−0.448	79	1.09	20.0	6.2
Water	−0.428	−0.420	98	0.60	14.1	9.3

The D_0 and k_s values are averaged from a set of experimental values.

hemin in aqueous surfactant solutions are reported. The diffusion coefficient and the rate of heterogeneous electron transfer of hemin in aqueous surfactant micelles are measured.

Experimental

Hemin (bovin) and Triton X-100 were from Sigma Chemical Co., USA, sds, $NMe_3(C_{16}H_{33})Br$, tris(hydroxymethyl)aminoethane (Tris buffer) and tetramethylammonium bromide from Merck, UK. Sodium nitrate was recrystallised from doubly distilled water. Hemin and Triton X-100 were used without further purification, sds was purified by recrystallisation twice from ethanol–water and $NMe_3(C_{16}H_{33})Br$ by recrystallisation from acetone. Different micellar solutions were prepared[7,12] by dissolving the surfactant (4 g for 4% solution) in deionised and distilled water (100 cm^3) containing 0.1 M NMe_4Br. The solution was adjusted to pH 8.0 (Tris buffer). The resulting suspension was warmed at 50 °C to get a clear solution of micelles at room temperature. The 4% stock solution of the micelles was diluted by an aqueous solution of pH 8.0 buffer (Tris) containing 0.1 M NMe_4Br in order to get surfactant solutions of varying concentration. A slightly alkaline solution of 0.1 mM hemin chloride was added to each of the micellar solutions of varying concentration at pH 8.0 and the mixtures allowed to equilibrate in the dark at 40–50 °C for about 1 h. On cooling, clear solutions were obtained. The final concentration of heme in the micellar solution was *ca.* 1 mM. Samples prepared in this way obeyed Beer's law over a wide range of hemin concentration. Iron(II) hemes were prepared by reduction with dithionite under an inert atmosphere in a Thurnberg apparatus. The optical spectra were recorded on a Hitachi (model U-3210) spectrophotometer.

Electrochemical measurements were performed on a BAS 100B electrochemical analyser (Bio-Analytical system, USA) using a three-electrode cell assembly with nitrogen gas purging lines. A glassy-carbon disc was used as working electrode and Ag–AgCl (3 M aqueous NaCl) electrode as a reference. The working electrode was polished using a 0.1 μM alumina and diamond slurry (BAS polishing kit) followed by sonication in a ultra-sonicating bath. The potential of the reference electrode was periodically checked. The voltammograms were plotted on a Fujitshu FPG-300 plotter. A background voltammogram of the surfactant solutions containing 0.1 M NMe_4Br and 0.1 M $NaNO_3$ at a glassy-carbon electrode showed that the micelles were free from redox interferences in the potential range of interest.[20] The sds and Triton X-100 surfactant solutions offer convenient media for potential scanning in the range +0.6 to −1.5 V (*vs.* Ag–AgCl), while in $NMe_3(C_{16}H_{33})Br$ micelles the convenient potential range was +0.6 to −1.5 V (*vs.* Ag–AgCl).

The ionic strength of the solution was 0.1 M. The supporting electrolyte was 0.1 M $NaNO_3$ at about 100 times the concentration of the electroactive species; *IR* compensation was made in a very few cases. The scan rate dependence of the cyclic voltammograms was studied in the range 20 to 1000 mV s^{-1}. The values of $E_{\frac{1}{2}}$ obtained were confirmed by OSWV. All the measurements of pH dependence of the midpoint potential

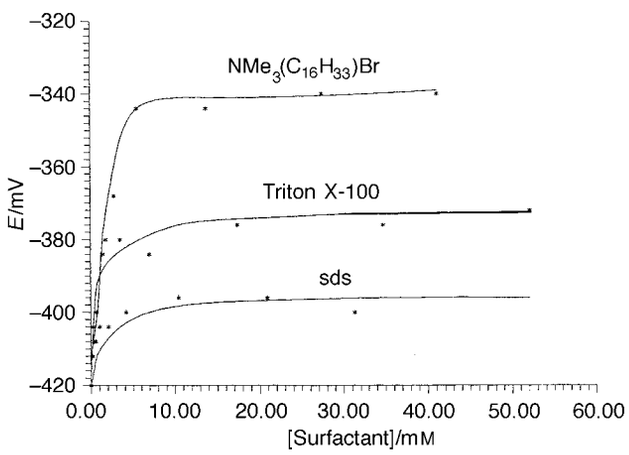


Fig. 1 Change of $E_{\frac{1}{2}}$ with surfactant concentration (10^{-3} M hemin at pH 8.0, 0.05 M Tris buffer) †

were performed by OSWV. This method has an edge over conventional pulse voltammetry because the time taken to complete the experiment is much shorter. In this work the square-wave amplitude was 25 mV, the frequency 15 Hz and the potential step height for base staircase wave form 4 mV. The diffusion coefficients were measured by chronoamperometry.[20,21] The effective surface area of the working electrode was measured by the same technique using a compound with known diffusion coefficient (potassium ferrocyanide, $D_0 = 0.63 \times 10^{-5}$ cm^2 s^{-1}). Heterogeneous electron-transfer rate constants were measured from the ΔE_p values at several scan rates following Nicholson's procedure.[22]

Results and Discussion

The results of the electrochemistry experiments on hemin in aqueous sds, Triton X-100 and $NMe_3(C_{16}H_{33})Br$ surfactants are shown in Table 1. Values at pH 8.0 are shown so as to compare them with those in aqueous solutions where the hemin is soluble at alkaline pH. The influence of the micellar environment on the midpoint potentials is clearly observed. The $E_{\frac{1}{2}}$ values reported here correspond to the Fe^{III}–Fe^{II} couple[14] in [FeL].

Effect of surfactant on midpoint potential

Fig. 1 shows the midpoint potential of the Fe^{III}–Fe^{II} couple as a function of concentration of various surfactants. The midpoint

† The reported c.m.c. values of surfactants in neutral solutions in the absence of electrolytes at 25 °C are 8.1×10^{-3}, 9.2×10^{-4}, 4.0×10^{-4} M in sds, $NMe_3(C_{16}H_{33})Br$, and Triton X-100, respectively.[10,23] These values are sensitive to temperature and for ionic surfactants depend on the presence of electrolytes in solution.[23] The c.m.c. values calculated from Fig. 1 are approximately 7×10^{-3}, 16×10^{-4} and 5×10^{-4} M, respectively. Since the $E_{\frac{1}{2}}$ is very sensitive to the concentration of $NMe_3(C_{16}H_{33})Br$ below its c.m.c., and since the error in measurement is large (*ca.* ±10 mV), the c.m.c. values in $NMe_3(C_{16}H_{33})Br$ could not be accurately measured.

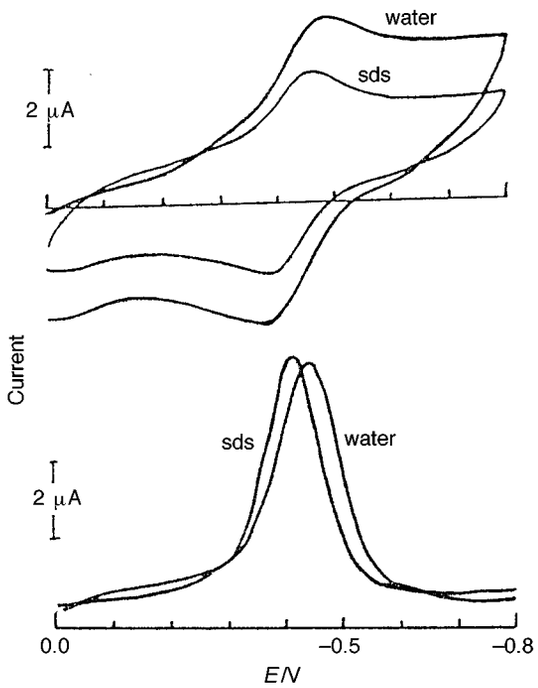


Fig. 2 Cyclic voltammograms and Osteryoung square-wave voltammograms of 1.0 mM hemin in pure water and sds micelles (buffer 0.05 M Tris, 0.1 M $NaNO_3$)

potential of hemin in water is *ca.* −0.42 V (*vs.* Ag–AgCl). Gradual addition of surfactant to the aqueous solution induces an anodic shift which continues to increase until the surfactant concentration reaches a maximum value close to the critical micellar concentration (c.m.c.) of the surfactant.[10,23] Above the c.m.c. the redox potentials assume a constant value. Except for $NMe_3(C_{16}H_{33})Br$, the c.m.c. values calculated from Fig. 1 agree well with those reported;[23] the expected trend in the values are found, Triton X-100 < $NMe_3(C_{16}H_{33})Br$ < sds.

The electrochemical behaviour of hemin in aqueous sds micelles is shown in Fig. 2, and the results are summarised in Table 1. The pH of the solution was maintained at 8.0 in a well buffered medium. The midpoint potentials are quite sensitive to the pH variation. In the absence of a buffer the anodic peaks are considerably broadened. A surfactant concentration of 4%, which is much above the c.m.c.,[10,23] was chosen so as to ensure complete micellisation. Under these conditions the midpoint potentials are independent of any minor fluctuation in surfactant concentration during electrochemistry experiments.

At pH 8.0 the aquahydroxo hemin species is the only electroactive species present in aqueous surfactant solution. The optical spectrum of 10^{-5} M hemin in sds micelles shows λ_{max} at 400, 490, 523 (sh) and 600 nm which indicates the presence of the aquahydroxo species.[7,12] In the presence of 4% sds (Fig. 2) the peak separation is 73 mV and the $i_{pc}/i_{pa} = 1.0$. The $E_{\frac{1}{2}}$ of the Fe^{III}–Fe^{II} couple in sds is found at −0.411 ± 0.004 V (*vs.* Ag–AgCl). A plot of i_{pc}/i_{pa} *versus* the square root of the scan rate is linear (Fig. 3). In the presence of 4% $NMe_3(C_{16}H_{33})Br$ (Fig. 4) the peak separation is 77 mV and $i_{pc}/i_{pa} = 1.07$. The $E_{\frac{1}{2}}$ of the Fe^{III}–Fe^{II} couple in $NMe_3(C_{16}H_{33})Br$ is found at −0.312 ± 0.008 V (*vs.* Ag–AgCl). A plot of i_{pc}/i_{pa} as a function of the square root of the scan rate is linear (Fig. 3).

Hemin in micelles is quite stable to hydrolysis (to μ-oxo hemin) and aggregation. Reproducible results are obtained for the complex in micellar solution recorded as a function of time (multiple cycle CV) or when the experiment is repeated several times. However in pure water or in aqueous ethanol the results are not as consistent. The voltammogram in sds micelles and the scan-rate dependence of the peak currents are indicators of quasi-reversible behaviour. The cyclic voltammograms in micelles are less complicated by associated chemical reactions

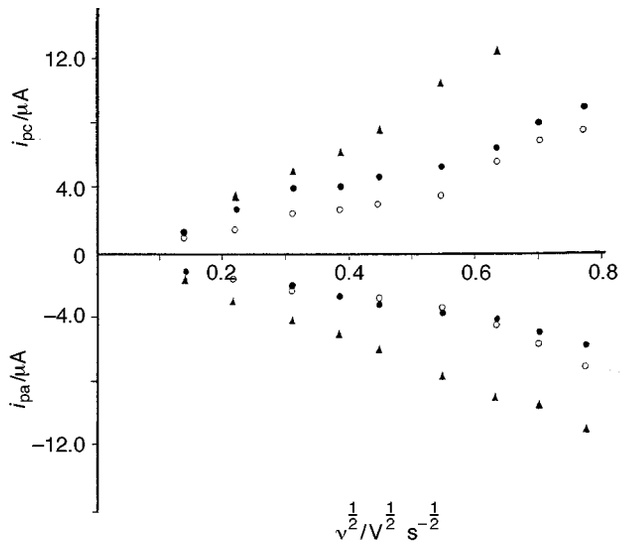


Fig. 3 Plots of i_{pc} and i_{pa} *vs.* the square root of the scan rate for 10^{-3} M hemin at pH 8.0 (buffer 0.05 M Tris) encapsulated in sds (○), Triton X-100 (●) and $NMe_3(C_{16}H_{33})Br$ (▲) micelles

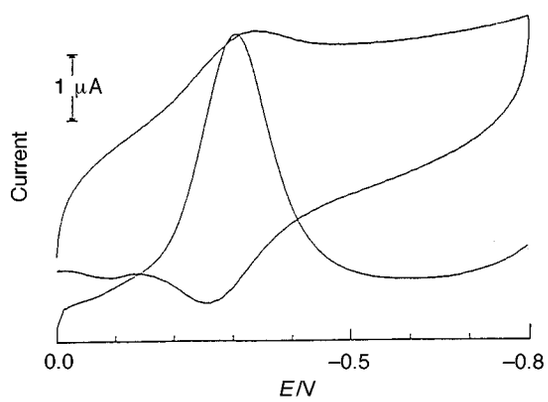


Fig. 4 Cyclic voltammogram and Osteryoung square-wave voltammogram of 1.0 mM hemin in 4% $NMe_3(C_{16}H_{33})Br$ (0.05 M Tris, 0.1 M $NaNO_3$)

(coupled chemical reactions) preceding or following the electron-transfer process. Since both hydrolysis to form the μ-oxo hemin species and aggregation require intermolecular interaction, micelle encapsulation would prevent such processes much better than in water or in aqueous ethanol media.

From a double potential-step chronocoulometry experiment, a plot of charge (Q) *versus* $t^{\frac{1}{2}}$ was linear (not shown) with an intercept (background corrected) of 1.75 μC for hemin in water. The large intercept for the reverse step indicates[20,24] that the reduced hemin is strongly adsorbed at the glassy-carbon electrode. However, a chronocoulogram of hemin in aqueous sds (Fig. 5) shows a charge *vs.* time response typical of a diffusion controlled process. Analysis of the data[24] yielded an intercept of *ca.* 0.7 μC for hemin in aqueous micelles, indicating that the adsorption at the electrode surface is significantly less in the micelles.

The change in the midpoint potential of the hemin complex in various surfactants is shown in Fig. 6. The potentials, within the micelles, vary (anodically) in the order: EtOH–water < sds < Triton X-100 < $NMe_3(C_{16}H_{33})Br$. The average anodic shift of the potential of hemin in aqueous micelles with respect to water is *ca.* +20 mV in sds, *ca.* +60 mV in Triton X-100 and *ca.* +120 mV in $NMe_3(C_{16}H_{33})Br$. The large anodic shift indicates that iron(III) hemin is easier to reduce, hence iron(II) hemes are more stable, in aqueous micellar solutions with respect to water.

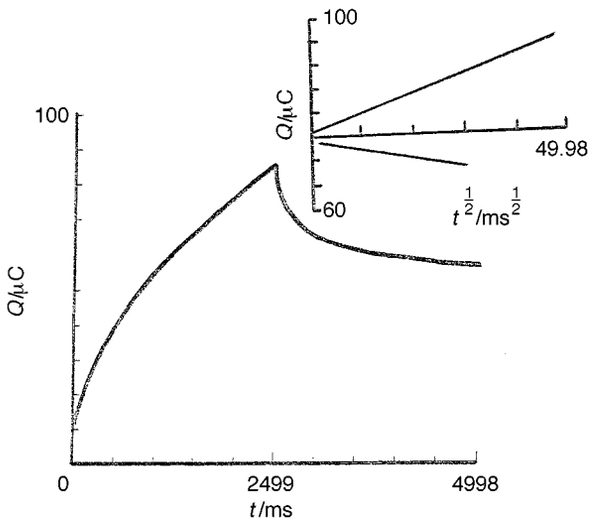


Fig. 5 Charge *vs.* time response of double potential-step chronocoulometry of 1 mM hemin in 4% aqueous sds. The Anson plot is obtained as output from a BAS 100B electrochemical analyser

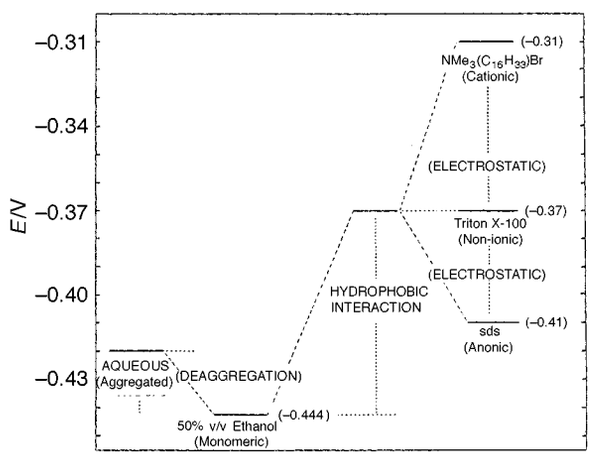


Fig. 6 Representation of $E_{\frac{1}{2}}$ values of hemin in different media. The experimental data are reported here by assuming that the net hydrophobic effect on the redox potential is the same in $NMe_3(C_{16}H_{33})Br$, sds and Triton X-100 micelles. Within the micelles the potentials are assumed to be predominantly due to the electrostatic influence of surface charges.

Diffusion coefficient and rate of heterogeneous electron transfer

The diffusion coefficients of the hemin complex in the micelles $D_0 = (1.5–5.3) \times 10^{-7}$ cm^2 s^{-1} are an order of magnitude smaller than that for hemin in aqueous ethanolic media, $D_0 = (1.2–2.0) \times 10^{-6}$ cm^2 s^{-1} (Table 1). This may be due to an increase in the effective size and high viscosity of the micellar solutions, which retard the diffusion of the encapsulated electroactive species.[20]

The value of the diffusion coefficient of aqueous solutions of monomeric hemin in various surfactants and ethanolic media increased in the order $NMe_3(C_{16}H_{33})Br$ < Triton X-100 < sds. Comparison between the various surfactants shows that the diffusion rate of the entrapped iron complex to the electrode is largest in the sds micelles.

The rates of the heterogeneous electron transfer are found to be smaller in the micelles as compared to those in aqueous ethanolic media. The observed rate constants follow the order sds < Triton X-100 < $NMe_3(C_{16}H_{33})Br$ < EtOH–water. The heterogeneous electron-transfer rate constants of hemin in aqueous micelles are much smaller than those for monomeric or dimeric hemes in aqueous solution [16] and comparable to that of cytochrome c ($k_s = 1.7 \times 10^3$ cm s^{-1}).

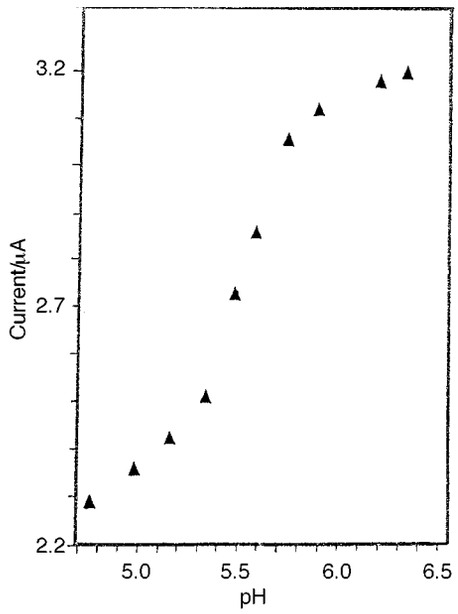


Fig. 7 Plot of peak current of the square-wave voltammogram of hemin (1 mM) in 4% aqueous sds as a function of pH

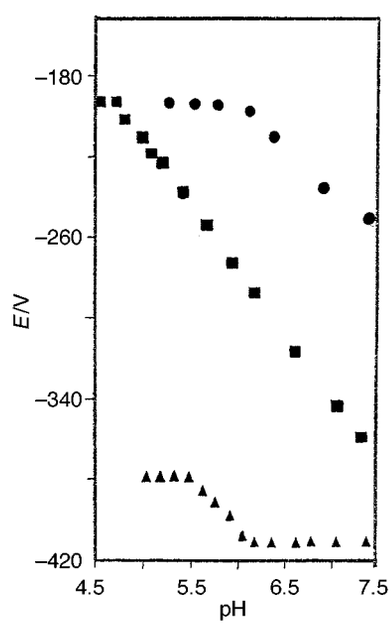


Fig. 8 The pH dependence of the midpoint potential of hemin (1 mM) encapsulated in aqueous $NMe_3(C_{16}H_{33})Br$ (●), sds (▲) and Triton X-100 (■) micelles. The midpoint potentials ($E_{\frac{1}{2}}$) were measured by OSWV at a glassy-carbon electrode *vs.* Ag–AgCl ($I = 0.1$ M $NaNO_3$)

Dependence of midpoint potential on pH

Hemin in aqueous micelles show an aqua–hydroxo equilibrium (1) with a pK_a of 6.1 in $NMe_3(C_{16}H_{33})Br$, 4.7 in Triton X-100

$$[FeL(H_2O)_2]^+ \cdot \text{micelle} \rightleftharpoons [FeL(OH)(H_2O)] \cdot \text{micelle} + H^+ \quad (1)$$

and 5.5 in sds micelles.[7] This was confirmed by measurement of the absorbance at 393 nm as well as the peak currents of voltammograms (OSWV) as a function of pH (Fig. 7). The proton uptake/release at the axial ligand has a considerable influence on the NMR spectrum of diaqua hemin in aqueous sds micelles.[12]

The dependence of the midpoint potential on pH is shown in Fig. 8. The variation of $E_{\frac{1}{2}}$ with pH is very small when the operating pH is much less than the pK_a value. When the operating pH is above the pK_a value the midpoint potential shifts

cathodically as the pH increases. The change in the potential per unit change in pH, $\Delta E/\Delta pH$, is −58 mV in $NMe_3(C_{16}H_{33})$-Br, −59 mV in Triton X-100 and −59 mV in sds micelles. This indicates proton-coupled electron transfer[4] involving one proton and one electron, equation (2). Here the uptake/release

$$[Fe^{III}L(OH)(H_2O)] + e^- + H^+ \rightleftharpoons [Fe^{II}L(H_2O)_2] \quad (2)$$

of protons at the co-ordinated OH/H_2O controls the redox potential of the heme.

Comparison with proteins

In an ordinary aqueous solution the redox potential of hemin is large and negative; on increasing the concentration of surfactant the potential shifts to more positive values (Figs. 1 and 6). In a surfactant micelle the hemin is located in the hydrophobic region near the micelle water interface,[8] where the effective relative permittivity[25] ($\varepsilon = 32$) is much lower than that in bulk water. Thus a change from an essentially aqueous to an essentially non-polar local heme environment in the micelles leads to a positive shift of the midpoint potential. This work provides experimental evidence to support the hypothesis[4,5] that a hydrophobic local heme environment of a apoprotein may be responsible for the large positive redox potential of heme proteins. Since the micelles are more dynamic (with rapid on-off equilibrium of the amphiphiles), the hydrophobic effect on the redox potential is expected to be much smaller than that in proteins. The maximum positive shift of +120 mV between a heme in $NMe_3(C_{16}H_{33})Br$ micelles and that in water is much smaller than the positive shift of +400 mV between cytochrome c and a model heme complex in an aqueous medium.[5] The difference in the shifts may be attributed to a lower value of the effective relative permittivity of the local heme environment in aqueous micelles as compared to that in protein pockets.[4,5]

The redox potential of hemin in an aqueous micellar solution is dependent on the state of protonation of the axial ligand. There are several proteins where the uptake/release of protons at an ionisable functional group controls the redox potential of the heme. Thus hemin in aqueous surfactant micelles is a good model with which to study proton-coupled electron transfer in hemoproteins.

Conclusion

Aqueous surfactant micelles are excellent media for electrochemical studies of iron porphyrins. Monomeric hemes may be studied by electrochemical techniques in aqueous solutions under conditions similar to those in hemoproteins.

The electrostatic and hydrophobic influence of the micelles on various electrochemical parameters such as the redox potential of the Fe^{III}–Fe^{II} couple in iron porphyrins, the diffusion coefficient and the heterogeneous electron-transfer rate constant can be measured. Comparison of the midpoint potentials shows that there is a large anodic (positive) shift in the micelles as compared to that in ordinary aqueous solutions. Within the micelles, the anodic shift (*i.e.* difference of midpoint potential of hemin in micelles and in water or in ethanolic aqueous media) follows the trend sds < Triton X-100 < $NMe_3(C_{16}H_{33})$-Br. The apolar nature of the local heme environment which solubilises and stabilises the iron(II) hemes in the micelles may be responsible for the large anodic shifts with respect to water or aqueous ethanol. Electrostatic interaction of the surface charges introduce an additional shift of the midpoint potential; a positive surface charge on the micelle gives the largest anodic shift.

The electron transfer at the iron site of hemin is controlled by uptake/release of protons at the axially co-ordinated H_2O/OH ligand. A change in the midpoint potential per unit change of pH of *ca.* −59 mV indicates proton-coupled electron transfer in micelle-encapsulated hemes.

Acknowledgements

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