$$\cot\theta_H = \alpha T^2 + b \qquad (4)$$

as indeed observed by Chien *et al.*[1] in Zn-doped YBCO single crystals, and subsequently reported more widely[2–7].

The above argument is consistent with the absence of anomaly when the Hall current is out-of-plane (i.e. along the *c*-axis) with the crossed-fields in-plane. This is because the *c*-axis transport has been shown to be controlled intrinsically by the in-plane transport in the normal state, and hence there is a single relaxation time $\tau_{tr} \propto T^{-1}$ consistent with the T-linear in-plane resistivity[9–11].

We would like to conclude with the following comments. The main point of our argument is that while the system may be a strongly correlated one with non-Fermi liquid-characteristics, e.g. the T-linear in-plane resistivity, a small deformation (in the sense of distribution function) of the system when probed appropriately may behave differently, and in particular as a normal Fermi-liquid. This notion is, of course, somewhat familiar in terms of the idea of the electron- or the hole-pockets of a complex Fermi-surface representing sub-sets of carriers with different characteristics, e.g. effective masses, etc. In our case of the Hall angle, the electric field prepares the small deformation (sub-set of carriers) and the magnetic-field probes it. It should be possible to extend this argument to other, possible multipolar, deformations.

Note added in proof: In response to the clarification sought by the referee in his report, received at the proof stage, I clarify once again that in this work we do not introduce explicitly any specific modification of the collision term subsumed in the treatment of B. G. Kotliar, A. Sengupta and C. M. Varma (*Phys. Rev.*, 1996, **B53**, 3573–3577) as a dissipative force proportional to magnetic field and acting sideways, arising from a singular skew-scattering mechanism. Instead, we have decomposed the Hall response as a two-step process, and have distinguished the two relaxation rates in terms of the natures of the respective deformations of the distribution functions involved and the reference distributions to which these deformations relax. This crucial point is totally missed in the usual Boltzmann transport equation that balances the effect of the force $[\mathbf{E}+\mathbf{V}\times\mathbf{H}]$ against the collision integral.

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Direct electrochemistry of heme undecapeptide in aqueous surfactant solutions: The effect of hydrophobicity and axial ligation on redox potential of heme

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Direct electrochemistry of heme undecapeptide (microperoxidase) in micellar solution has been achieved for the first time. Results show a smooth increase in the $E_{1/2}$ with increase in surfactant concentration. The diversity of redox potentials of the heme in various redox hemeproteins is an important topic of investigation in recent years. The origin of this diversity can either be variation in axial ligation or hydrophobicity of the heme pocket in different hemeproteins. We report here that association of heme undecapeptide (microperoxidase) with aqueous detergent micelles can indeed modulate the redox potential of the heme to a sizable extent. Micellar environment mimics the hydrophobicity of the protein cavity. Thus, our results demonstrate on how the hydrophobicity can tune the redox potentials of hemeproteins.

ELECTROCHEMICAL techniques are recognized[1] as powerful means to characterize electron transfer properties of chemical and biological systems. Several workers have reported[2] electrochemical studies on metalloproteins using mediators such as ferrocenes and methylviologen. Direct electron transfer between the protein and the electrode has recently attracted extensive interest and several studies on 'direct' electrochemistry of metalloproteins have been reported[2]. Thus, direct application of cyclic voltammetry to the study of redox processes of various metalloproteins is achieved in presence of suitable pro-

moters (with no redox activity in the potential of study) to understand the mechanism of electron transfer between the electrode and the redox center[1,2]. The redox potential of the heme has been shown to vary over a large range from a positive value (e.g. +740 mV in the ferryl complex in cytochrome c peroxidase[3], +260 mV in cytochrome c[4]) to a negative value (e.g. −450 mV in naked heme in aqueous solution[5]). The variation of axial ligation to iron in the heme has been shown to account for a part of the differences in redox potentials[6]. However, the large difference in redox potentials of the heme site with the same coordination geometry (e.g. −270 mV in horseradish peroxidase[7,8] and +50 mV in myoglobin[8,9], both having the same ferric protoheme complex with axial histidine and water ligands) cannot be explained by consideration of only ligand-induced effects on redox potential. The importance of hydrophobicity of the protein environment on the redox potential of heme in hemeproteins was proposed by Brunori and coworkers[10], however, any experiment demonstrating the role of the protein environment in modulating the redox potential has not been reported so far.

Direct electrochemical investigations on heme undecapeptide (microperoxidase) obtained by hydrolysis of horse heart cytochrome c have earlier been reported by Santucci *et al.*[10] in aqueous buffer. Microperoxidase is an iron complex of porphyrin c covalently linked by thioether bridges to two cysteine residues (Cys 14 and Cys 17 of native horse heart cytochrome c) in an undecapeptide[11]. The effect of hydrophobicity on the redox potential of heme in cytochrome c can thus be conveniently studied using microperoxidase. Aqueous detergent micelles have been shown to encapsulate the heme and have earlier been used to study the effect of hydrophobicity on the stability and electronic properties of the heme complex[12,13]. We have carried out direct cyclic voltammetric studies on microperoxidase in absence and in presence of different surfactant solutions and report our results here. The effect of hydrophobicity on the redox potential of the heme complex has been demonstrated for the first time using microperoxidase complexes in various surfactant solutions with different charge and chain lengths. The effect of axial ligation on the redox potentials of monomeric micelle encapsulated heme peptide has also been reported.

Microperoxidase was obtained from Sigma, USA and was purified by passing through Sephadex G10 column in 0.05 M sodium phosphate buffer, pH 7.4. Direct electrochemical studies on microperoxidase was carried out at room temperature (298 K) in a small three-electrodes cell with a platinum grid as counter electrode, a saturated calomel electrode as the reference and glassy carbon disk as the working electrode. The supporting electrolyte used for most of the experiments was 20 mM $MgSO_4$. Electrochemical studies were carried out using an EG & G PAR Potentiostat Galvanostat (model M273A) interfaced with an IBM PC. The potential values were reported with respect to the normal hydrogen electrode (NHE). The surfactants used were cetyl trimethyl ammonium bromide (CTAB), dodecyl trimethyl ammonium bromide (DTAB), sodium dodecyl sulphate (SDS), sodium cholate, sodium deoxy cholate, Triton X-100, Tween 80 obtained from Sigma and were used without further purification.

Well defined electrochemistry of microperoxidase (~ 0.2 mM) was obtained in aqueous buffer and in surfactant solutions. Quasi-reversible voltammetric waves ($\Delta E \approx 60$ mV) were obtained with $I_C/I_A \approx 1.05$, and peak width ~ 125 mV (see Figure 1 *a*, *b*). The $E_{1/2}$ value of microperoxidase in aqueous buffer solution was found to be −164 (±10) mV corresponding to the Fe^{3+}/Fe^{2+} redox couple, which is in good agreement with the previous report[10]. The peak current was found to be proportional to the square root of the scan rate (up to 200 mV/sec), indicating the diffusion-controlled nature of the electron transfer between the electrode and microperoxidase[14].

Cyclic voltammetry of microperoxidase was carried out in presence of different concentrations of the surfactants. Direct cyclic voltammetry of microperoxidase was also carried out in presence of cyanide and imidazole both in presence and in absence of surfactants. The $E_{1/2}$ value of microperoxidase was found to shift towards positive value on increasing surfactant concentration. Further, the magnitude of this shift was found to depend on the hydrophobicity of the micelles formed by the surfactants. Figure 1 *a* shows typical dc voltammograms of microperoxidase in different concentrations of DTAB. The peak current was found to decrease at very large ($\gtrsim$ 20 mM DTAB) surfactant concentration possibly because of association of the peptide with the micelles leading to decrease in diffusion rate. The $E_{1/2}$ value was found to increase with increasing concentration of surfactant until it reached a maximum value at a certain high surfactant concentration close to the critical micellar concentration (CMC) of the micelle[15]. A similar trend was observed for all surfactants studied in the present report, but the extent of the maximum positive shift at the saturation point was different for different surfactants. Figure 1 *b* shows typical cyclic voltammograms of microperoxidase obtained at high concentrations of CTAB and DTAB (the cyclic voltammogram of the peptide in aqueous buffer is plotted for comparison). Figure 2 shows a plot of the observed $E_{1/2}$ against surfactant concentration, which shows an increase in the $E_{1/2}$ value of microperoxidase with increasing surfactant concentration. The magnitude of the positive shift of the $E_{1/2}$ value increases in the following order: deoxycholate < cholate < SDS < DTAB < CTAB. The hydrophobicity of the surfactant has been shown to depend

on the chain length, surface charge and CMC of the surfactant micelles[15]. The hydrophobicity of the surfactant increases approximately in the same order[15] (i.e. deoxycholate < cholate < SDS < DTAB < CTAB) as the positive shift of $E_{1/2}$ of microperoxidase observed in the present study. This indicates that the difference in hydrophobicity exerted by the surfactants possibly gives rise to the different positive shifts of the $E_{1/2}$ value of microperoxidase.

Cyclic voltammetric experiment was performed on microperoxidase in presence of cyanide and imidazole as axial ligands at different concentrations of surfactants. $E_{1/2}$ values of microperoxidase–imidazole and microperoxidase–cyanide complexes were found to be – 205 mV and – 190 mV respectively which matched with previous reports[8,10]. The $E_{1/2}$ values in both these complexes were found to increase with increase in surfactant concentration showing similar trend as that in microperoxidase.

Table 1 gives the shifts in $E_{1/2}$ of microperoxidase due to association with micelles and/or axial ligation by cyanide and imidazole. Table 1 shows that the hydrophobicity of the DTAB micelles causes a positive shift of ~ + 60 mV in the $E_{1/2}$ of microperoxidase in presence of any axial ligand. Microperoxidase exists as dimer with a *N*-terminal valine residue (Val 11) or lysine residue (Lys 13) of one molecule coordinated to the iron of the other[12] and micelle encapsulation of the peptide involves breaking of the axial amino acid–Fe bond and subsequent ligation of –OH at ambient pH. The contribution of various axial ligands towards the $E_{1/2}$ value was calculated by simple difference between two pairs of potentials as shown in Table 1. The relative contributions of different axial ligands (with respect to -OH) to the $E_{1/2}$ value of microperoxidase estimated by this empirical method follows the order: H_2O (+ 50 mV) > OH (0) > Val/Lys (– 44 mV) > CN (– 70 mV) > imidazole (– 100 mV). The contribution of hydrophobicity of CTAB (100 mV), DTAB (60), cholate (15 mV) and deoxycholate (~ – 6 mV) was also estimated by this method.

The results demonstrate that increase in hydrophobicity of the medium can cause a large positive shift (up to + 100 mV in CTAB) of the redox potential of the heme. A smooth increase in $E_{1/2}$ of microperoxidase with increase in surfactant concentration indicates that the aggregation of the surfactant with the peptide takes place by association of increasing number of surfactants with a peptide molecule until micelle is formed. The exact

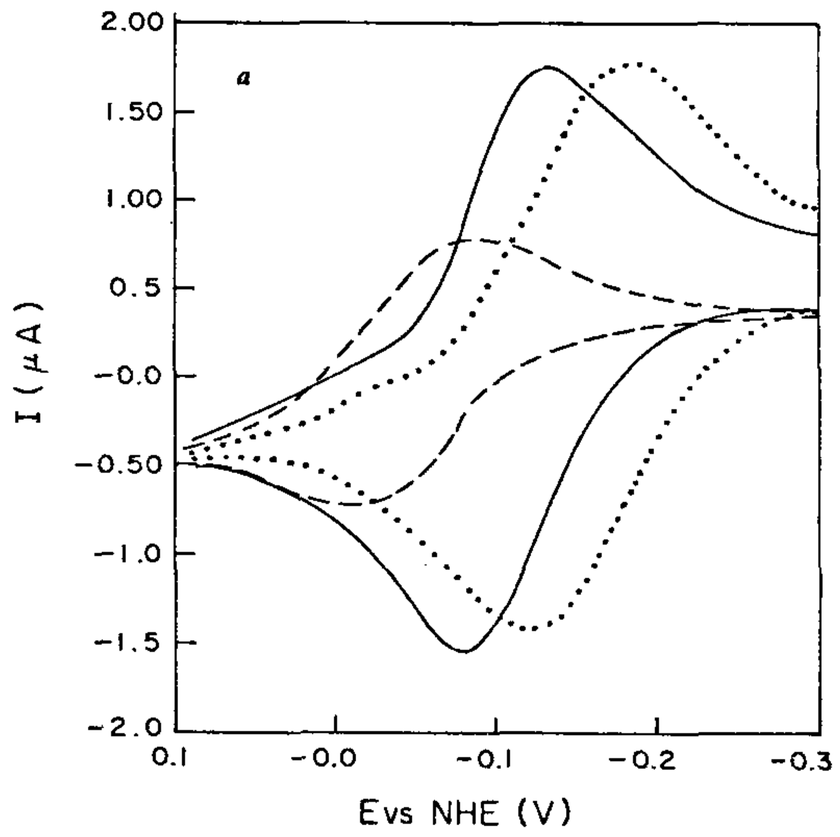

Figure 1 *a*. Typical dc cyclic voltammetry of microperoxidase (0.2 mM) in presence of different amounts of surfactants in 20 mM $MgSO_4$ and phosphate buffer (0.5 M) pH 7.4. In buffer solution (. . . .); in presence of 0.6% DTAB (——); and in presence of 1.8% DTAB (– – –).

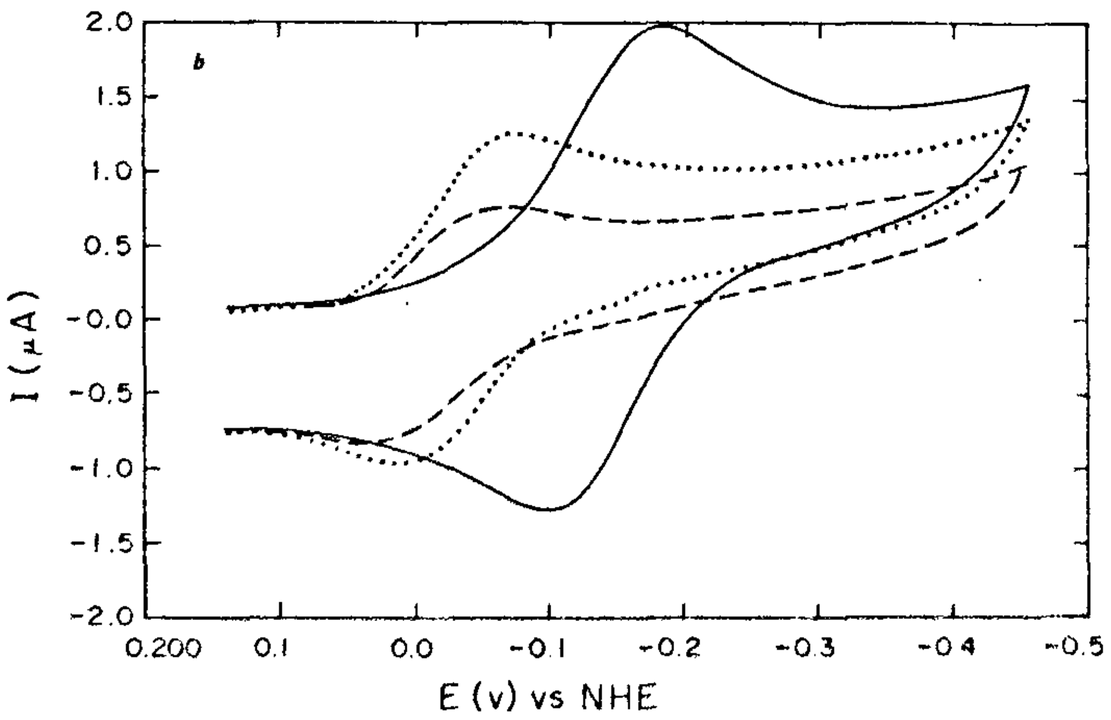

Figure 1 *b*. DC cyclic voltammetry of microperoxidase (0.2 mM) in absence of surfactant (as in 1 *a*) (——); in presence of 1.5% DTAB (. . . .); and in presence of 0.2% CTAB (– – –). Scan rate 20 mV/sec.

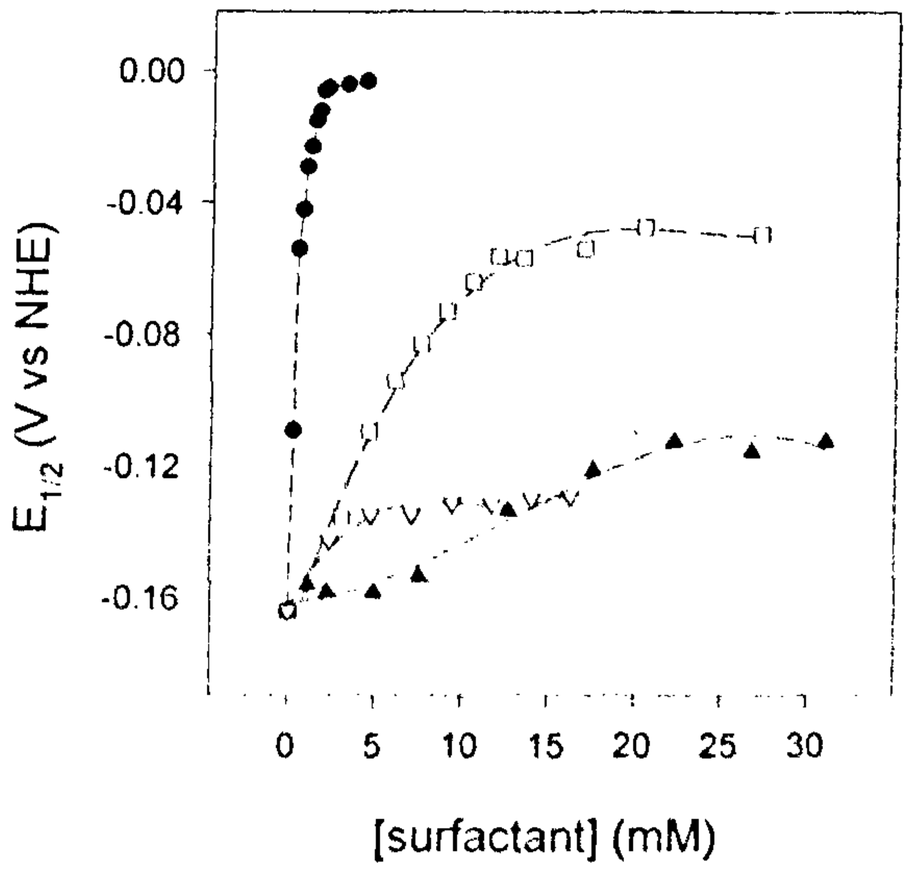

Figure 2. Plot of observed $E_{1/2}$ (vs NHE) of microperoxidase against surfactant concentrations. (– • – • – • –) in CTAB; (– □ □ □ –) in DTAB; (–V–V–V–) in deoxycholate and (–▲–▲–▲–) in cholate solutions (experimental conditions same as in Figure 1).

Table 1. Change in the $E_{1/2}$ of various complexes of microperoxidase in aqueous and surfactant solutions

	Ligand	Medium	$E_{1/2}$ mV		$\Delta E_{1/2}$ mV	Effects*
E_1	Val/Lys	Aq. buffer pH 7.4	–164	–	0	Val/Lys†
E_2	OH	DTAB solution	–60	$E_2 - E_1$	+104	Hyd + OH – Val/Lys
E_3	CN	Aq. buffer pH 7.4	–190	$E_3 - E_1$	–26	CN–Val/Lys
E_4	CN	DTAB solution	–130	$E_4 - E_3$	+60	Hyd
E_5	Imidazole	Aq. buffer pH 7.4	–205	$E_5 - E_1$	–45	Imidazole–Val/Lys
E_6	Imidazole	DTAB solution	–150	$E_6 - E_5$	+55	Hyd
E_7	H_2O	Aq. buffer pH 3.5**	–70	$E_7 - E_1$	+94	H_2O–Val/Lys
E_8	H_2O	DTAB solution	0	$E_8 - E_7$	+70	Hyd

Hyd: Hydrophobicity;
*Effect of ligation/hydrophobicity;
**pH = 3.5 in acetate buffer; Microperoxidase exists as dimer with a *N*-terminal valine residue of one molecule coordinated to the iron of the other;
†The dimeric form of microperoxidase has been shown to contain axial ligand either Val 11 or Lys 13 apart from the His at ambient pH in aqueous solution.

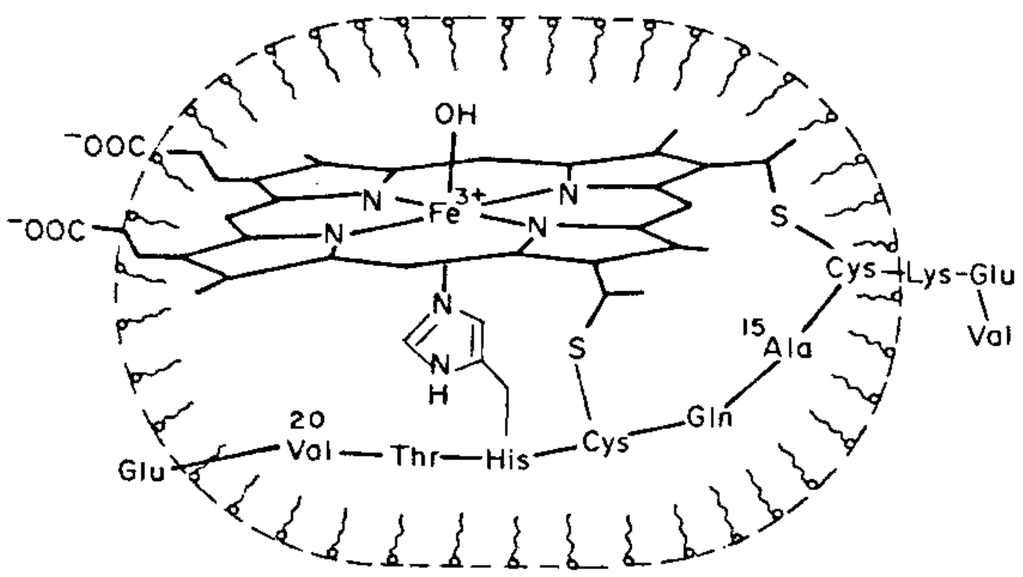

Figure 3. Possible structure of microperoxidase bound to micelles. The ionic groups of the peptide would possibly reside near the surface of the micelle while the highly aromatic heme ring would preferentially occupy the hydrophobic region of the micelle.

structure of the micelle-bound peptide is not known, however earlier studies[12] showed that simple hemes preferentially reside in the hydrophobic region near the interface of the micelle. Figure 3 shows a possible structure of the peptide–micelle aggregate. The micellar environment mimics the hydrophobicity of the protein cavity[12]. Thus the present study indicates that the redox potential of hemeproteins is influenced not only by the axial ligation by different amino acid residues but also by the protein environment surrounding the heme site and the magnitude of the shift caused by the protein environment can be equally important as the axial co-ordination to the heme in determining its biochemical redox behaviour, which agrees with the earlier proposition of Santucci *et al.*[10].

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