Electrochemistry of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO}')_4]^{6-}$ in liposomes and microemulsions

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$[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO}')_4]^{6-}$, a structural analogue of active center of ferredoxin and P cluster of nitrogenase, shows two distinct reversible couples with redox potential values $-643\pm5$ mV and $-972\pm5$ mV vs. Ag-AgCl due to $3+/2+$ and $2+/1+$ couples of the core $[\text{Fe}_4\text{S}_4]$ respectively in liposomes. Two redox linked protonation sites, with $pK_{ox}^{a}$ at 3.2 with corresponding $pK_{red}^{a}$ at 4.3 due to carboxylate and another $pK_{red}^{a}$ at 5.8 due to thiopropionate $S$ shift the redox potential of $3+/2+$ couple by 200 mV. In microemulsion containing negative sds molecules (ME-sds) the redox potentials due to $3+/2+$ and $2+/1+$ couples are $-670$ mV and $-920$ mV ($pH=4.6$) while these values are $-544$ mV and $-800$ mV respectively ($pH=5.5$) versus Ag-AgCl in microemulsion containing positive ctab molecules (ME-ctab). The trend in diffusion coefficient of the cluster in different medium is found to be: ME-sds>ME-ctab>liposomes.

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Electro transfer is a fundamental reaction in life processes. Fe-S clusters, being active center analogue of iron sulfur proteins play an important role in basic life processes like respiration, photosynthesis and nitrogen fixation. Stability of the Fe-S clusters in different oxidation states, and hence their redox potential, is crucial in controlling the electron transfer behaviour of the iron sulfur protein. The redox potential is influenced by a number of factors like hydrophobicity of the microenvironment around the active center, number of hydrogen bonds (H-bond) around the active center and presence of ionisable functional group attached to the cluster.

Iron sulfur clusters with thiolate ligands are well known active center analogue of iron sulfur proteins. Redox potential of these clusters are far more negative than the redox potential of iron sulfur proteins. This was attributed initially to the absence of H-bonding between amide hydrogen of peptide chain and sulfur of the Fe-S core and/or of the terminal cysteine residue ligated on the iron atoms in the ferredoxins. Recent studies have shown that nature of microenvironment around the active center plays a major role in determining the redox potential. Odell and Geary have reported that synthetic iron sulfur cluster undergoes 220 mV anodic shift in redox potential in presence of albumin in water as compared to that in water alone. Very recently we have shown that $[\text{Fe}_4\text{S}_4]$ cluster shows a 220 mV positive shift compared to that in organic solvent, while inside positive surfactant film on electrode surface.

Redox linked protonation/deprotonation is observed in natural Fe-S proteins–NADH–quinone oxidoreductase, Reiske iron sulfur proteins, nitrogenase, Azobecter vinelandii etc. The pH dependence of redox potential of a number of model systems have revealed $pK_{ox}^{a}$ at 5.8-6.7 and $pK_{red}^{a}$ at 7.3-9.3 with a slope of $-60$ mV/pH due to protonation of bridged S of Fe-S core. Recently, we have reported that protonation of the carboxylate in $\text{Fe}_4\text{S}_4$ core can impart a positive shift of 140 mV.

Microemulsions are thermodynamically stable, isotropic and optically clear dispersion of oil-in-water or water-in-oil of droplet dimension 50-500 Å and have been used as medium of organic reactions for decades. Liposomes are bi-layer phospholipid vesicles, with distinct hydrophobic and hydrophilic regions, mimicking cell membranes, and have been used to study various electron transfer systems including proteins across bi-layer membranes.

Herein, report the electron transfer behaviour of active center analogue of ferredoxin, $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO}')_4]^{6-}$, in microemulsions and liposomes. The redox linked protonation of the cluster in liposome due to carboxylate is also reported.
Materials and Methods

All the chemicals were from Merck and were used without any further purification. C H Instrument (USA) electrochemical analyzer with three electrode cell assembly with N₂ gas purging lines was used for electrochemical studies. The working electrode was platinum with Ag-AgCl (3 M NaCl) as reference. In Osteryoung square wave voltammetry (OSWV), the wave square wave amplitude was 25 mV, the frequency 15 Hz and potential for base staircase wave front was 4 mV.

The [Fe₄S₄(SCH₂CH₂COO)₄]⁶⁻ was prepared as reported using Schlenk technique under nitrogen environment. The electronic spectra recorded for the cluster in mercaptide buffer at pH 8.6 show peaks at λ_max = 290 nm and λ_max = 380 nm. The reported spectrum of the complex in mercaptide buffer at pH 9.2 was similar with peaks at λ_max = 300 nm and λ_max = 400 nm.

Liposomes were prepared by injection method as reported. Two microemulsions were used, which we shall designate as ME-sds and ME-ctab as they contain negative SDS and positive CTAB surfactant molecules respectively. The composition of the two microemulsions are; ME-sds: 82.1 g water, 3.2 g cyclohexane, 4.9 g SDS, 9.8 g 1-butanol; and ME-ctab: 28.0 g water, 5.0 g n-hexane, 37.0 g CTAB, 30.0 g 1-butanol and were prepared as earlier reported. The cluster was added to 10 mL of the liposome or micro emulsion solution and allowed to equilibrate in dark at 45°C for 30 min under nitrogen environment. The final concentration of the cluster was ca. 1 mM. For redox potential versus pH studies, the pH was varied using mercaptopropionic acid and 0.1 M potassium hydroxide.

Results and Discussion

The cyclic voltammogram of the cluster in liposome at pH 6.0 and scan rate 100 mVs⁻¹ is shown in Fig. 1. Two distinct reversible couples due to [Fe₄S₄(SCH₂CH₂COO)₄]⁶⁻/⁷⁻ and [Fe₄S₄(SCH₂CH₂COO)₄]⁷⁻/⁸⁻ are observed with redox potentials -643±5 mV and -972±5 mV respectively versus Ag-AgCl reference. The redox potential values are further confirmed by the OSWV measurements (Fig. 2). The ΔE_p values for the couples are found to be 180 mV and 150 mV respectively and are much less than the ΔE_p value (320 mV) for the cluster in mercaptide buffer indicating better electrochemical reversibility of the cluster in liposome compared to that in mercaptide buffer.

Figure 3 shows the cyclic voltammogram of the cluster in microemulsion ME-sds at different scan rates. Two redox couples due to [Fe₄S₄(SCH₂CH₂COO)₄]⁶⁻/⁷⁻ and [Fe₄S₄(SCH₂CH₂COO)₄]⁷⁻/⁸⁻ are observed with redox potential values -670 mV and -920 mV at scan rate 100 mVs⁻¹ with ΔE_p values 125 mV and 60 mV respectively (pH=4.6). These redox potential values are found to be within 5mV, with that measured from OSWV measurements. The plot of cathodic and

![Fig. 1—Cyclic voltammogram of [Fe₄S₄(SCH₂CH₂COO)₄]⁶⁻ in liposome at pH = 6.0 (mercaptide buffer) and scan rate = 100 mVs⁻¹. [WE: Pt, RE: Ag-AgCl, Supporting electrolyte: 0.1 M NaClO₄.](Image 1)

![Fig. 2—Osteryoung Square Wave Voltammogram of [Fe₄S₄(SCH₂CH₂COO)₄]⁶⁻ in liposome at pH = 6.0 (mercaptide buffer) and scan rate = 100 mVs⁻¹. [WE: Pt, RE: Ag-AgCl, supporting electrolyte: 0.1 M NaClO₄.](Image 2)
anodic current versus square root of scan rate for the 

\([Fe_4S_4(SCH_2CH_2COO)_4]^{6/7^-}\) couple is linear with no intercept, indicating that the electron transfer is diffusion controlled. In microemulsion ME-ctab also good reversible cyclic voltammogram is obtained (Fig. 4) for the two redox couples with mid-point potential values at –544 mV \((\Delta E_p = 190 \text{ mV})\) and –800 mV \((\Delta E_p = 180 \text{ mV})\) respectively \((pH = 5.5)\). Thus in ME-ctab which contains positive CTAB molecules the redox potential due to \([Fe_4S_4(SCH_2CH_2COO)_4]^{6/7^-}\) and \([Fe_4S_4(SCH_2CH_2COO)_4]^{7/8^-}\) couples are respectively ca 115 mV and 120 mV more positive compared to those in ME-sds containing negative SDS molecules. The presence of positive CTAB molecules around the cluster influences the cluster to be in more negative state making the reduction processes easier and hence resulting in positive potential. On the other hand, in ME-sds the negative SDS molecules make the more negative state less stable, making reduction difficult, thus resulting in more negative redox potential.

The double potential step chronocoulometric study is done for the cluster in all the medium using a 250 mV pulse for 25 m s. The charge versus (time) \(^{1/2}\) plot are found to be linear for both the reverse step and forward step. The diffusion co-efficient of the cluster onto the electrode surface in reduced state are found to be \(0.48\times10^{-10}, 0.29\times10^{-10}\) and \(0.24\times10^{-8} \text{ cm}^2\text{s}^{-1}\) in liposome, ME-ctab and ME-sds respectively. Thus, the diffusion co-efficient is \(10^2\) times higher in ME-sds than in ME-ctab and liposome. This trend is in agreement with the trend recently reported by us for some other ferredoxin active center model system\(^{12}\). The electrostatic repulsion between the negative cluster and negative polar head groups of the SDS molecules of ME-sds helps the diffusion and hence highest diffusion coefficient results.

The pH dependence of redox potential

The redox potentials due to both the redox couples for the cluster have been measured as a function of \(pH\). The redox potential of the couple at more positive potential that is \([Fe_4S_4(SCH_2CH_2COO)_4]^{6/7^-}\) couple, is found to change with \(pH\) while the other couple, \([Fe_4S_4(SCH_2CH_2COO)_4]^{7/8^-}\), is found to be independent of \(pH\). The reason behind this independency of later redox couple on \(pH\) is not clear to us. The redox potential of the former couple of the cluster was measured by OSWV at a Pt working electrode in the \(pH\) range 2.5-6.0 in liposomes and in the range 4.0-7.0 in microemulsions.

In liposomes, the redox potential of the couple starts shifting in the negative direction at \(pH\) 3.2 and continues to shift till \(pH\) 5.8 and thereafter remains constant. The net shift is ~200 mV with a slope of ~120 mV indicating two independent redox linked proton coupled electron transfer events\(^{20}\). Such characteristic \(pH\) dependence of the redox potential may be interpreted in terms of protonation/deprotonation equilibria involving one of the four carboxylates of the oxidized cluster \([Fe_4S_4(SCH_2CH_2COO)_4]^{6^-}\) as well as of the reduced \([Fe_4S_4(SCH_2CH_2COO)_4]^{3^-}\) one as shown in Scheme 1. \(R, T\) and \(F\) being gas constant, temperature and Faraday constant, respectively. \(K^{\text{ox}}\) and \(K^{\text{red}}\) are expressed as Eqs 1 and 2.

![Cyclic voltammogram of \([Fe_4S_4(SCH_2CH_2COO)_4]^{6^-}\) in SDS emulsion (ME-sds) at \(pH = 4.6\) (mercaptide buffer) and scan rate = 20, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700 and 800 mVs\(^{-1}\). \([\text{WE: Pt, RE: Ag-AgCl, supporting electrolyte: 0.1 M NaClO}_4]\).](image)

![Cyclic voltammogram of \([Fe_4S_4(SCH_2CH_2COO)_4]^{6^-}\) in CTAB emulsion (ME-ctab) at \(pH = 5.5\) (mercaptide buffer) and scan rate = 100 mVs\(^{-1}\). \([\text{WE: Pt, RE: Ag-AgCl, supporting electrolyte: 0.1 M NaClO}_4]\).](image)
$K_{\text{ox}} = \frac{[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO}^-)_{4}]^{6-}}{[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO})_{4}H]^{5-}} \ldots (1)$

$K_{\text{red}} = \frac{[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO}^-)_{4}]^{7-}}{[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO})_{4}H]^{6-}} \ldots (2)$

where $K_{\text{ox}}$ and $K_{\text{red}}$ are the acid dissociation constants of the protonated species in oxidized and reduced state respectively. $E_{1/2}^{1}$ and $E_{1/2}^{2}$ are the redox potentials of the couples of unprotonated and protonated species, respectively. The redox potential for the overall electrode reaction can be written as Eqs 3 or 4.

$E_{1/2}^{1} = E_{1/2}^{1} - \frac{RT}{nF} \ln \frac{K_{\text{ox}} (K_{\text{red}} + [H^+])}{K_{\text{red}} (K_{\text{ox}} + [H^+])} \ldots (3)$

$E_{1/2}^{2} = E_{1/2}^{2} - \frac{RT}{nF} \ln \frac{([K_{\text{red}} + [H^+])(K_{\text{ox}} + [H^+]])}{... (4)}$

A positive shift in $E_{1/2}$ with $[H^+]$, implies that $K_{\text{ox}}$ is larger than $K_{\text{red}}$. Hence according to Eq 3, $E_{1/2}^{1}$ approaches $E_{1/2}^{1}$ when $[H^+] < < K_{\text{red}}$ and according to equation 4, $E_{1/2}^{2}$ approaches $E_{1/2}^{2}$ when $[H^+] > > K_{\text{ox}}$. A least squares analysis of the data in the pH range 2.5 - 4.5 using Eqs 3 or 4 shows that computer simulated curve fits well for $n = 1$ with $pK_{\text{ox}}$ and $pK_{\text{red}}$ at 3.2 and 4.3 respectively. A similar analysis of the data for the cluster in microemulsions yields $pK_{\text{ox}}$ at 4.5, $pK_{\text{red}}$ at 5.4 in Me-SDS and $pK_{\text{ox}}$ at 4.6, $pK_{\text{red}}$ at 5.6 in Me-CTAB. Recently, we have reported that this cluster in different micelles shows $pK_{\text{ox}}$ at 2.5-3.2 due to one of the four carboxylates. As the $pK_{\text{ox}}$ of the cluster due to bridged S is at pH above 7.4, in the pH range $pK_{\text{ox}}$ to $pK_{\text{red}}$ the cluster is protonated at one of the four core S. Hence, protonation/deprotonation of carboxylate may influence the redox potential via formation of H-bonding between protonated core S and the carboxylate as shown in Fig. 5. This H-bonding could bring about a partial deprotonation leading to stabilization of the oxidized state by charge compensation with a cathodic shift of mid-point potential of the cluster as the pH increases.

In micro emulsions, the values of $pK_{\text{ox}}$ are ca. 1.3 and 1.4 pH value higher in Me-SDS and Me-CTAB respectively compared to those in liposomes, implying that protonation of the carboxylate is easier when the cluster is in micro emulsions. The presence of significant amount of organic molecules in micro

![Diagram](Scheme_1.png)

Fig. 5—Formation of possible H-bonding between the protonated core S and one of the three nearby carboxylates of $[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO}^-)_{4}]^{6-}$ at pH $> pK_{\text{red}}$ and the protonated form at a pH $< pK_{\text{ox}}$. 
emulsions probably helps the carboxylate to be in non-ionic protonated form at relatively higher pH.

J L F Duff et al. proposed Eq 5 to explain redox potential versus pH profile of a system where protonation takes place only in the reduced state.

\[ E = E^\circ + RT/nF \ln \left[ 1 + \left( dH^+ / K_{red} \right) \right] \]  \hspace{1cm} (5)

This equation may also be written as Eq 6 below:

\[ E_{1/2} = E^\circ + RT/nF \ln \left[ 1 + ([H^+] / K_{red}) \right] \]  \hspace{1cm} (6)

\( E^3 \) is the limiting redox potential of the cluster in reduced state and on further protonation in addition to the protonation justified by Eqs 3 and 4. \( E_{1/2} \) will approach \( E^3 \) when \( K_{red} \gg H^+ \). Nakamoto et al. have reported that protonation on the terminal S gives rise to a \( pK_{red} \) at 5.85. The least squares fitting of the data (pH 4.5-6.0) to Eq 6, for the cluster in liposomes, shows that the best fitted line is for \( n=1, y=1 \) with \( pK_{red} \) at 5.8 confirming the protonation/deprotonation event involving the terminal S. The presence of \( pK_{red} \) without its corresponding \( pK_{ox} \) is not a rare redox feature of Fe-S cluster. In Me-SDS and Me-CTAB, the redox potential becomes close to the potential of the other couple, whose redox potential is independent of pH, and both the OSWV peaks merge to give a broad peak making detection of redox potential difficult beyond pH 7.0.

The electron transfer role of ferredoxins is controlled by the redox potential of Fe-S cluster which is influenced by the properties of the micro environment of the medium such as hydrophobicity, pH, etc. Nitrogenases which convert nitrogen into ammonia, accomplish the transformation by a sequence of coupled electron and proton transfer reactions. There is a \([\text{Fe}_{14}S_4]\) cuboidal unit in Fe-part of nitrogenase and this unit transfers electrons from external reductant to the MFe-protein (where M=Mo, V or Fe) part of nitrogenase. The MFe-protein part is tetramer of the type \( \alpha_4\beta_4 \) with each \( \alpha_2\beta_2 \) subunit having two structurally unique Fe-S clusters, one P cluster and one Fe-Mo cluster. The P cluster, which has Fe and S only, acts as storage of proton and electrons for conversion of di-nitrogen into ammonia. The results presented herein, which elaborate the effect of micro environment on (i) redox potential, and, (ii) electron coupled protonation/deprotonation site of \([\text{Fe}_{14}S_4(SCH_2CH_2COO)^4]\) \( \text{cluster should help understand the role ferredoxin and nitrogenase.}

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References