Redox linked protonation/deprotonation on the carboxylate of \([\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO})_4]^{6-}\) in aqueous micellar solutions

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\[\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO})_4]^{6-}\], which acts as active center analogue of ferredoxin, demonstrates a proton coupled electron transfer phenomenon with \(pK_a^{ox}\) at 3.0, 2.5, and 3.2 and \(pK_a^{red}\) at 4.7, 4.3, and 4.8, in 3\% (w/v) aqueous SDS, TX-100 and CTAB micelles. When the operating \(pH\) is between \(pK_a^{ox}\) and \(pK_a^{red}\), the observed redox Bohr effect exhibits a 60 to 70 mV per \(pH\) unit slope indicating one proton per electron coupled event. These low \(pK_a\) values arise because of the protonation/deprotonation of one of the four carboxylates. Also, protonation of the carboxylate leads to 120-140 mV positive shift in the mid-point potential. Formation of H bonding between the carboxylate and the neighbouring protonated core S is proposed as reason for the very low \(pK_a\).

Ferredoxins and other iron sulfur proteins form a comprehensive class of non-heme iron proteins which act as electron carriers in a number of important biological processes. The electron carrying properties of these proteins are related to the ability of their iron sulfur active sites to exist in several different oxidation levels interrelated by one-electron transfer reactions. Redox linked protonation/deprotonation has been observed in the natural Fe-S proteins NADH-quinone oxidoreductase at cluster N2, the reiske iron sulfur protein, the P-cluster of nitrogenase, and Azobacter vinelandii ferredoxin I, AvFdi.

Iron sulfur clusters with thiolate ligands have been synthesized and studied as model of the active center of iron sulfur proteins. The water-soluble clusters are found to exhibit more positive redox potential than water insoluble clusters in organic solvents. However, the redox potential of the former is still more negative than that of 4-Fe ferredoxins. This negative redox potential of synthetic models was attributed to the absence of hydrogen bonding formed between amide hydrogen of the peptide chain and sulfur of the Fe₄S₄ core and/or of the terminal cysteine residue ligated on the iron atoms in the ferredoxins. Recent studies have shown that the microenvironment around the active center is more important than the number of NH—S hydrogen bonds around the active center. Theoretical studies have revealed that solvent exposure plays a major role in determining the electrostatic environment and thereby the redox potential of [Fe₄S₄] cluster. For example, compared to ferredoxins in high potential iron sulfur proteins (HiPIP), the Fe₄S₄ cluster is enclosed by several hydrophobic aromatic side chains that restrict solvent accessibility and hence gives more positive redox potential. In accordance with this, Odell and Geary have reported that synthetic iron-sulfur cluster undergo 220 mV anodic shift in redox potential in the presence of bovine serum albumin in water compared to that in water alone.

The redox linked protonation of ferredoxin active center analogues is reported in aqueous micellar and protein medium. In case of [Fe₄S₄(SR)_4] where R= n-C₆H₁₂ and C₆H₄-p-t-Bu, the protonation/deprotonation was observed to take place on the S atom of Fe₄S₄ core with oxidized \(pK_a^{ox}\) at ca. 5.8-6.7 and reduced \(pK_a^{red}\) at ca. 7.3-8.8. Kennedy and Gibney, recently reported \(pH\) dependence of redox potential of a designed protein, IGA-[Fe₄S₄], with oxidized and reduced \(pK_a\) at 6.5 and 9.3, respectively with a slope of 60 mV/pH. The electrochemical studies of [Fe₄S₄(SCH₂CH₂COO)₄]⁺ cluster have been carried out in protein environment and a \(pK_a\) value at 7.4, reported to be due to protonation of bridged S of the Fe-S cluster. However, the role of the carboxylate in controlling the redox potential of [Fe₄S₄] core through protonation/deprotonation has yet not been investigated till now.

We report here a new proton coupled electron transfer site due to the protonation/deprotonation of carboxylate of the cluster, [Fe₄S₄(SCH₂CH₂COO)₄]⁺, in three different surfactant micelles. It is found that only one out of the four carboxylates is involved and this affects the redox potential significantly.

**Experimental**

The [Fe₄S₄(SCH₂CH₂COO)₄]Na₃(NBu₄) (NBu₄: Tetrabutylammonium) cluster is prepared as
reported\textsuperscript{29}, using Schlenk technique under nitrogen environment. All the chemicals except triton X-100 (SIGMA) are from Merck and distilled twice before use. Dissolved oxygen is removed by passing dry nitrogen gas for half an hour. The surfactants used are cetyltrimethylammonium bromide (CTAB), triton X-100 (TX-100) and sodium dodecylsulphate (SDS). These have cationic, neutral and negative polar head groups, respectively. Surfactant solutions used are 3\% (w/v) in mercaptide buffer at pH 9.2 and prepared as reported. The cluster is added to 10 mL of the micellar solution in mercaptide buffer and then allowed to equilibrate in dark at 45°C for 30 min in nitrogen environment. The concentration of the cluster in micellar solution is 1 mM. pH was varied using mercaptopropanoic acid and 0.1 M potassium hydroxide.

BAS (Bioanalytical system) 100 W electrochemical analyzer with a three-electrode cell assembly was used in the present investigations. Cyclic voltammetry (CV) was used to measure the mid-point potentials, which were further confirmed by the Osteryoung Square Wave Voltammetry (OSWV) technique. CV and OSWV experiments were carried out under a blanket of nitrogen gas after passing the gas through the solution for ten min. The working electrode was a platinum rod, reference electrode was Ag-AgCI and sodium nitrate (0.1 M) was the supporting electrolyte. Concentration of the cluster in solution for measuring mid-point potential was kept at 1 mM. In the OSWV experiments, the square wave amplitude was 25 mV, the frequency 15 Hz and the potential step height for base step case wave front 4 mV. Background voltammograms of the surfactant solutions at the platinum electrode show that they are free from any redox interferences in the potential range of interest. The working electrode was cleaned by polishing with 0.1 \(\mu\)M alumina using a polishing kit (BAS) followed by sonication before each run.

Results and discussion

The electronic spectra recorded for the cluster \([\text{Fe}_4\text{S}_4\text{(SCH}_2\text{CH}_2\text{COO')}_4]\text{Na}_4(\text{NBu}_4)\) in mercaptide buffer at pH 8.6 show peaks at \(\lambda_{\text{max}} = 290\) nm and \(\lambda_{\text{max}}\) (sh) = 380 nm (Fig. 1). The reported spectrum\textsuperscript{29} of the complex in mercaptide buffer at pH 9.2 is similar with peaks at \(\lambda_{\text{max}} = 300\) nm and \(\lambda_{\text{max}}\) (sh) = 400 nm. However, the electronic spectrum of the ferredoxin isolated from rhodospirillum rubrum reported\textsuperscript{3} to show peaks at \(\lambda_{\text{max}} = \text{ca. } 290\) nm and \(\lambda_{\text{max}}\) (sh) = ca. 390 nm.

Figure 2 shows the cyclic voltammogram of the cluster in 3\% (w/v) SDS and TX-100 micelles at pH 4.5 and 3.5, respectively at scan rate 100 mVs\textsuperscript{-1}. Good reversible cyclic voltammogram was obtained in case of 3\% (w/v) CTAB solution too at pH=4.0 (not shown). Our earlier work has shown that this surfactant concentration is far above the cmc values and at this surfactant concentration, the background voltammogram is free from any redox interferences\textsuperscript{30,31}. One reversible redox couple is observed with redox potential value at -737 mV, -700 mV and -690 mV in SDS, TX-100 and CTAB, respectively versus Ag-AgCl (3 M NaCl) as reference.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig1}
\caption{Electronic spectra of \([\text{Fe}_4\text{S}_4\text{(SCH}_2\text{CH}_2\text{COO')}_4]\text{Na}_4(\text{NBu}_4)\) in mercaptide buffer at pH 7.6.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{Fig2}
\caption{Cyclic voltammogram of \([\text{Fe}_4\text{S}_4\text{(SCH}_2\text{CH}_2\text{COO')}_4]\text{Na}_4(\text{NBu}_4)\) in 3\% (w/v) SDS and TX-100 micelles at pH 4.5 and 3.5, respectively at scan rate 100 mVs\textsuperscript{-1}. Good reversible cyclic voltammogram was obtained in case of 3\% (w/v) CTAB solution too at pH=4.0 (not shown). Our earlier work has shown that this surfactant concentration is far above the cmc values and at this surfactant concentration, the background voltammogram is free from any redox interferences\textsuperscript{30,31}. One reversible redox couple is observed with redox potential value at -737 mV, -700 mV and -690 mV in SDS, TX-100 and CTAB, respectively versus Ag-AgCl (3 M NaCl) as reference.}
\end{figure}
NOTES

The redox potential values were further confirmed by OSWV experiments. This observed redox couple in surfactant micelles is due to the 6-17-couple\textsuperscript{21,29}. The $\Delta E_p$ values for the cluster are found to be 105, 130, and 120 mV in CTAB, TX-100, and SDS, respectively. The reported\textsuperscript{21} $\Delta E_p$ value of the cluster due to 6-17-couple is 320 mV in mercaptide buffer. Thus, compared to mercaptide buffer, the electrochemical reversibility of the cluster is better in aqueous micellar medium.

The mid-point potential of the cluster displays pH dependence between pH 2.5 to 5.0 in aqueous and different micellar medium as shown in Fig. 3. The redox potentials are measured by CV at a Pt working electrode versus Ag-AgCl as reference. The data are analysed, following the method described by Moore \textit{et al.}\textsuperscript{32} by a weighted non-linear least squares fit of the mid point potentials to a theoretical curve described by the Eq. (1):

$$E = E^o + (RT/nF) \ln \left( \frac{K_{a,\text{ox}}^\text{red} + [H^+]}{K_{a,\text{red}}^\text{ox} + [H^+]} \right)$$  \hspace{1cm} (1)

where $K_{a,\text{ox}}$ and $K_{a,\text{red}}$ are the acid dissociation constants of the protonated species $[\text{Fe}_{4}\text{S}_{4}\text{(SCH}_2\text{CH}_2\text{COO})]^{6+}$ and $[\text{Fe}_{4}\text{S}_{4}\text{(SCH}_2\text{CH}_2\text{COO})]^{5+}$, respectively, and $R$, $T$, and $F$ being the gas constant, temperature and Faraday constant, respectively. The best fitted theoretical curve corresponds to one proton and one electron ionization. In all the three micelles, both the oxidized and reduced $pK_a$, $pK_{a,\text{ox}}$ and $pK_{a,\text{red}}$ respectively, are observed and their values are given in Table 1. When the pH is lower than the $pK_{a,\text{ox}}$ and higher than $pK_{a,\text{red}}$ the redox potential found to remain constant. When the operating pH is between $pK_{a,\text{ox}}$ and $pK_{a,\text{red}}$, the observed redox Bohr effect exhibits a 60 to 70 mV per pH unit slope (Table 1) between $pK_{a,\text{ox}}$ and $pK_{a,\text{red}}$ indicating one proton per electron coupled event. The protonation of the carboxylate site in the cluster is found to impart a positive shift of 135, 120, 130 and 140 mV in mercaptide buffer, SDS, TX-100 and CTAB, respectively. The overall pH dependent redox behaviour of the cluster may be illustrated as shown in Scheme 1 ($E^o_{1/2}$ and $E^o_{2/2}$ are mid-point potentials below $pK_{a,\text{ox}}$ and above $pK_{a,\text{red}}$, respectively):

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|}
\hline
\textbf{Medium} & \textbf{$pK_{a,\text{ox}}$} & \textbf{$pK_{a,\text{red}}$} & \textbf{$\Delta E/\Delta pH$ (mV)} \\
\hline
Water & 2.7 & 4.5 & 65 \\
SDS & 3.0 & 4.7 & 60 \\
TX-100 & 2.5 & 4.3 & 65 \\
CTAB & 3.2 & 4.8 & 70 \\
\hline
\end{tabular}
\caption{$pK_{a,\text{ox}}$ and $pK_{a,\text{red}}$ of $[\text{Fe}_{4}\text{S}_{4}\text{(SCH}_2\text{CH}_2\text{COO})]^{6+}$ due to protonation/deprotonation of the carboxylate in different media [Surfactant concentration 3\% (w/v); WE: Pt, RE: Ag-AgCl; 3 M NaCl].}
\end{table}

Fig. 3—The pH dependence of the redox potential of $[\text{Fe}_{4}\text{S}_{4}\text{(SCH}_2\text{CH}_2\text{COO})]^{6+}$ and their best least square fitted theoretical curve in 3\% (w/v) aqueous mercaptide buffer, SDS, TX-100 and CTAB.
The \([\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO})_4]^{6-}\) cluster possesses two types of potential protonation sites: (i) the sulfur atoms of the cluster core, i.e., the inorganic sulfur (S) and sulfur of thiopropionic acid (S); and, (ii) the carboxylate group of thio propionic acid. Job and Brucie reported a \(pK_a\) of the cluster \([\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO})_4]^6\) at 7.4 in mercaptoe buffer and assigned it to the protonation of core sulfur. In TX-100 micelles, protonation on core S takes place in the clusters \([\text{Fe}_4\text{S}_4(\text{S-n-C}_6\text{H}_{13})_4]^{6}\) and \([\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_4-p-t\text{-Bu})_4]^{6}\) at pH 9.1 and 8.5, respectively which are much higher than the \(pK_a\) (s), we have observed. Further, the cluster \([\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2-p-t\text{-Bu})_4]^{6}\) in aqueous poly[2-(dimethylamino)hexanamide] (PDACA) solution was reported to undergo protonation at terminal S at pH 5.85 and core S at pH 8.8. From above examples, it is clear that the \(pK_a\) due to protonation of either core S or terminal S is much higher than the \(pK_a\) we have observed. Thus, the observed \(pK_a\) cannot be due to the protonation of the core or terminal S atoms and instead should be due to propionate.

Since the propionate carboxylate is three atom apart (one S and two C) from the iron, how can protonation/deprotonation of carboxylate influence mid-point potential of the cluster? Our earlier work on microperoxidase and iron porphyrins in aqueous surfactant micelles have shown that the \(pK_a\) due to the carboxylate, when H-bonded to co-ordinated water molecule, is at pH 2.5-3.0. The cluster \([\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO})_4]^{6}\) in the pH range 2.0 to 5.0 must be protonated at one core S since \(pK_a\) of core S is much higher than 5.0. Molecular scale model shows that this proton is quite capable of interacting with carboxylate of one of the three neighbouring mercapto propionic acids through H-bonding (Fig. 4) making it difficult to protonate the carboxylate and hence a very low \(pK_a\) is observed. This H-bonding could further bring about a partial deprotonation leading to stabilization of the oxidised state by charge compensation with a cathodic shift of mid-point potential of the cluster as the pH increases.

We have demonstrated that electrochemistry of \([\text{Fe}_4\text{S}_4(\text{SCH}_2\text{CH}_2\text{COO})_4]^{6-}\) can be carried out conveniently in aqueous as well as aqueous surfactant micelles even at a very low pH. The pH can have significant effect on the mid-point potential of the cluster through protonation/deprotonation of one of the thio propionates. The formation of H-bonding between the protonated core S and carboxylate of thio propionic acid is also indicated.

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