# Redox Behaviors of $(Bu_4N)_2[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]$ Solubilized in a Lecithin Membrane

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**Abstract:** Lecithin vesicles containing $[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]^{2-}$ ($[4\text{-Fe}]^{2-}$) in their lipid membranes form a stable thin layer on a hanging mercury drop electrode (HMDE); the $[4\text{-Fe}]^{2-}$ cluster in the membrane migrates with a diffusion coefficient of $8.8 \times 10^{-9}$ cm$^2$/s. Under acidic conditions, only the cluster adsorbed on the HMDE probably with two terminal sulfur atoms undergoes a reversible one-electron redox reaction due to the $[4\text{-Fe}]^{2-/3-}$ couple, whereas in alkaline media a normal electron transfer between $[4\text{-Fe}]^{2-}$ dissolved in the lecithin layer and the HMDE takes place together with the redox reaction due to the adsorbed species. The redox potentials of the clusters not only adsorbed on the HMDE but also dissolved in the lecithin layer shift by −55 mV/pH at 4 °C in the pH range 5.0–10.5, while those are essentially constant above pH 10.5, suggesting the existence of an equilibrium between the protonated and deprotonated clusters solubilized in lecithin membranes below pH 10.5.

Iron–sulfur proteins are widely distributed in living systems and play important roles in various biological redox reactions as electron-transfer catalysts, such as photosynthesis,[1] nitrate reduction,[2] nitrogen fixation,[3] and so on. Of those, low-potential 4-Fe ferredoxins exhibit their redox potentials near the equilibrium potential of the $H_2/H^+$ couple (−660 mV vs. SCE at pH 7.0 in water).[4] On the other hand, the redox potentials of synthetic $Fe_4S_4$ clusters in organic solvents are fairly negative compared with those of 4-Fe ferredoxins, and intrinsic and extrinsic factors influencing the redox potentials of synthetic $Fe_4S_4$ clusters have been well studied.[5,6] The $E_{1/2}$ values of the $[Fe_4S_4(SR)_4]^{2-/3-}$ (R = alkyl) and the $[Fe_4S_4(S\text{-}p\text{-}C_6H_4X)_4]^{2-/3-}$ (X = $NMe_2$, Me, H, $NO_2$) couples are largely dependent on electron-donating abilities of the substituents R or X; the spread of the $E_{1/2}$ value attains to 720 mV from the $[Fe_4S_4(S\text{-}t\text{-}Bu)_4]^{2-/3-}$ (−1.42 V vs. SCE) to the $[Fe_4S_4(S\text{-}p\text{-}C_6H_4NO_2)_4]^{2-/3-}$ couple (−0.70 V vs. SCE) in organic solvents.[5] It has been suggested that the redox potential of the $[Fe_4S_4(SR)_4]^{2-/3-}$ and $[Fe_4S_4(S\text{-}p\text{-}C_6H_4X)_4]^{2-/3-}$ couple in DMF is expressed by empirical eq 1 and 2 by employing the Taft $\sigma^*$ and Hammet $\sigma_p$ values, respectively.[5]

$$E_{1/2}\ (\text{V vs. SCE}) = 0.411\sigma^* - 1.30 \quad (1)$$

$$E_{1/2}\ (\text{V vs. SCE}) = 0.295\sigma_p - 1.04 \quad (2)$$

Solvents exhibit a remarkable effect on the redox potential as well; the $E_{1/2}$ value of synthetic water soluble $Fe_4S_4$ clusters, $[Fe_4S_4L_4]^{2-}$ (L = $SCH_2CH_2OH$, Cys(Ac)NHMe), is shifted continuously to negative potentials with an increase in the proportion of $Me_2SO$ in $H_2O$–$Me_2SO$ mixtures, and the value in $Me_2SO$ is 250–400 mV more negative than that in $H_2O$.[6] On the other hand, the $E_{1/2}$ value of *Clostridium pasteurianum* ferredoxin is essentially unchanged between $H_2O$ and $H_2O$–$Me_2SO$ mixtures unless otherwise the volume percent of $Me_2SO$ exceeds 40%,[6] where the protein may adopt its normal tertiary structure and the $Fe_4S_4$ core resides in a hydrophobic sphere of the protein. The anodic shift of $E_{1/2}$ (60–120 mV) of the 4-Fe ferredoxin, compared with a synthetic $Fe_4S_4$ cluster ligated with cysteine in $H_2O$–$Me_2SO$ mixtures with the range 0–40 vol %, has been ascribed to come from the tertiary structure of proteins.[6]

We have recently reported that $[Fe_4S_4(S\text{-}p\text{-}C_6H_4R)_4]^{2-}$ (R = $n$-$C_4H_9$, $n$-$C_8H_{17}$, and $n$-$C_{12}H_{25}$) solubilized in aqueous micellar solutions at pH 7.0 exhibits the redox potential of the (2-/3-) couple around −0.63 V vs. SCE, which is in the same region as the redox potentials of 4-Fe ferredoxins.[7] The study of the redox behavior of synthetic $Fe_4S_4$ clusters surrounded by hydrophobic spheres in water seems to be very important, since a number of Fe–S centers have been identified in membrane fractions of photosynthesis bacteria, blue-green algae, eukaryotic algae, mitochondria, and so on.[8] A lecithin bilayer membrane known as a major component of biomembrane may be superior to micelles as a model of membrane-bound Fe–S centers in electron-transport systems. This paper describes the redox behavior of $[Fe_4S_4(SC_6H_5\text{-}p\text{-}n\text{-}C_8H_{17})_4]^{2-}$ solubilized into a lecithin vesicle.

## Experimental Section

**General.** Commercially available guaranteed reagent grades of NaOH and $H_3PO_4$ were used without further purification. Egg yolk lecithin (biochemical use) was purchased from Wako Co. Ltd. and reprecipitated from $CHCl_3$–MeOH. The iron–sulfur cluster, $(Bu_4N)_2[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]$ ($(Bu_4N)_2$[4-Fe]), was prepared according to the literature.[7] Dimethylformamide (DMF) was purified by distillation from $CaH_2$ under reduced pressure and stored under $N_2$. All manipulations were carried out under $N_2$ atmosphere.

**Preparation of Lecithin Vesicles.** Lecithin vesicles were formed by ultrasonic dispersion of an aqueous suspension of lecithin as follows:[9] Lecithin (50 mg) was suspended in water (30 cm$^3$) at pH 6.0 (adjusted with 0.1 mol/dm$^3$ $H_3PO_4$–NaOH), and the suspension was ultrasonically irradiated with a 125 W Branson Sonifier (Model B-220) under $N_2$ at 4 °C for 60 min. The suspension was centrifuged at 18 000 rpm for 60 min in order to remove a small amount of lecithin aggregate. The resultant transparent solution was filtered through a 0.2 μm pore size filter (Milipore FGLO 2500), and the filtrate was treated on a Sephadex G-50 column (2 × 40 cm) with an $O_2$-free aqueous $H_3PO_4$–NaOH solution (0.1 mol/dm$^3$, pH 6.0) as an eluent (0.8 cm$^3$/min). A column effluent was monitored at 300 nm by a spectrophotometer equipped with a flow cell (Yanagimoto Co. Ltd., M-315).

**Solubilization of $[4\text{-Fe}]^{2-}$ in Lecithin Vesicles.** A given amount (0.003–0.45 cm$^3$) of the DMF solution of $(Bu_4N)_2$[4-Fe] ($1.40 \times 10^{-2}$ mol/dm$^3$) was injected into a stirred aqueous lecithin vesicle solution (30 cm$^3$) prepared at 4 °C, followed by sonication at that temperature for

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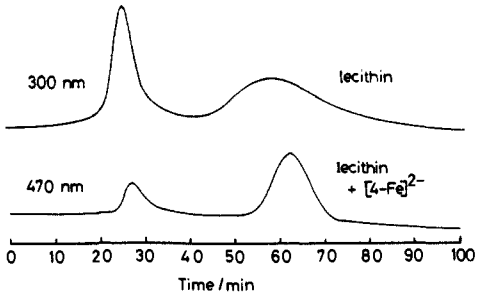


**Figure 1.** Elution patterns in Sephadex G-50 chromatograms of lecithin vesicles not containing and containing $[4\text{-Fe}]^{2-}$ in $H_2O$ at pH 5.20.

60 min under $N_2$. The resulting brown solution was chromatographed on a Sephadex G-50 column, and the effluent was monitored at the 460-nm band due to the CT transition of $[4\text{-Fe}]^{2-}$.

**Determination of the Lecithin Concentration.** The lecithin concentration of the vesicle solution was determined as $4.0 \times 10^{-3}$ mol/dm$^3$ by the literature method.[10]

**Cyclic Voltammetry.** Cyclic voltammograms were obtained by the use of a Hokuto Denko potentiostat HB-401, a Hokuto Denko function generator HB-107, and a Yokogawa Electric Inc. X-Y recorder 3077. The electrolysis cell was equipped with a Metrohm hanging mercury drop electrode (HMDE) E 410, a Pt auxiliary electrode, an SCE, and a nozzle for bubbling $N_2$. The voltage scan was started after the surface of a mercury drop (0.0187 cm$^2$) had been exposed to aqueous lecithin vesicle solutions for a given time ($t_{exp}$). The $E_{1/2}$ value[11] of $[4\text{-Fe}]^{2-}$ was measured in the pH range 5.0–11.5 at 4 °C. When the pH value was shifted by the addition of an aqueous NaOH or $H_3PO_4$ solution (0.1 mol/dm$^3$) to the lecithin vesicle solution, the pH values of the inner and outer aqueous phases of lecithin vesicles were equalized by sonication for 10 min at 4 °C.

## Results and Discussion

**Solubilization of $[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]^{2-}$ in Lecithin Vesicles.** Solubilization of $(Bu_4N)_2[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]$ ($(Bu_4N)_2[4\text{-Fe}]$) into a bilayer membrane of lecithin vesicles was confirmed from its chromatographic behavior on a Sephadex G-50 column. As reported elsewhere,[9] the elution of a lecithin vesicle solution prepared by sonication of an aqueous lecithin suspension for 60 min at 4 °C gives two bands with the retention times of about 24 and 56 min due to multi- and single-layer vesicles, respectively, when monitored at 300 nm, as shown in Figure 1. The $[4\text{-Fe}]^{2-}$-solubilized lecithin vesicles monitored at 470 nm, the CT band of $[4\text{-Fe}]^{2-}$, also give two bands of multi- and single-layer vesicles with almost the same retention times as the $[4\text{-Fe}]^{2-}$-free lecithin vesicles, respectively. This result indicates that the aggregation numbers of lecithin in multi- and single-layer vesicles are essentially unchanged before and after $[4\text{-Fe}]^{2-}$ was solubilized in their lipid membranes.

**Cyclic Voltammograms of $[4\text{-Fe}]^{2-}$ Solubilized in Lecithin Vesicles.** It is well-known that various surfactants in water and in water–alcohol mixtures rapidly aggregate to form an adsorbed layer on the surface of an electrode (relaxation time <500 ms),[12] and the resulting layer has more or less inhibitory effects on normal electron transfer redox reactions on the electrode.[13] Of a variety of surfactants, lecithin is known to form an especially stable thin layer on an Hg electrode. For example, the redox reaction of $Cd^{2+}$ on a hanging mercury drop electrode (HMDE) in water (0.45 mmol/dm$^3$) is inhibited almost completely in the presence of more than $3.90 \times 10^{-5}$ mol/dm$^3$ of lecithin.[14] It has been suggested that lecithin molecules adsorbed on an Hg electrode are considered

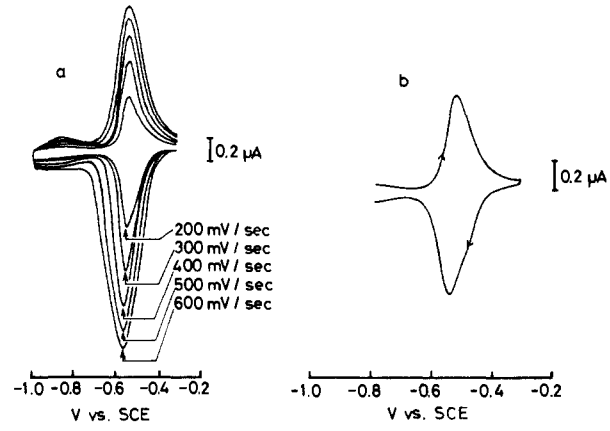


**Figure 2.** Cyclic voltammograms of $[4\text{-Fe}]^{2-}$ (10.3 μmol) solubilized in an aqueous lecithin vesicle solution (30 cm$^3$) at pH 5.65 after $t_{exp}$ = 1 min without (a) and with (b) applying –0.80 V vs. SCE to the HMDE for 1 min.

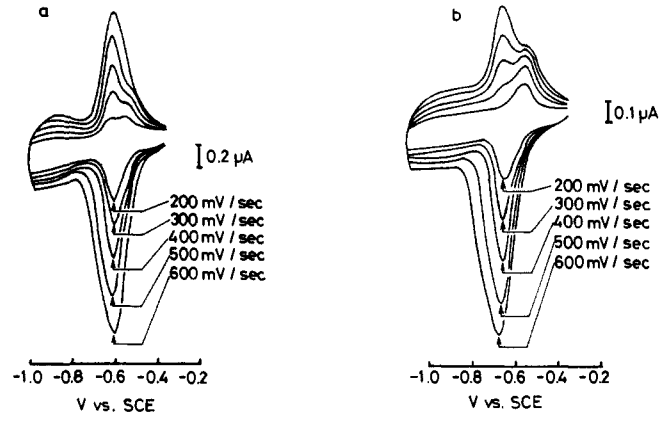


**Figure 3.** Cyclic voltammograms of $[4\text{-Fe}]^{2-}$ (10.3 μmol) solubilized in an aqueous lecithin vesicle solution (30 cm$^3$) at pH 6.72 (a) and pH 7.41 (b) at various sweep rates after $t_{exp}$ = 1 min.

to be arranged perpendicularly to the surface of the electrode to direct their polar head group toward water.[15] Despite such a strong inhibitory effect of lecithin molecules toward the redox reaction on an Hg electrode, the cyclic voltammogram (CV) of $[4\text{-Fe}]^{2-}$ solubilized in lecithin vesicles in water (pH 5.65) clearly shows a pair of cathodic and anodic waves due to the $[4\text{-Fe}]^{2-/3-}$ couple at –0.555 V vs. SCE after a new mercury drop of the HMDE was exposed to the solution for 1 min ($t_{exp}$ = 1 min), as shown in Figure 2a, which has essentially been unchanged even with stirring of the solution, while the peak currents of both cathodic and anodic waves are proportional to the sweep rates in the range 200–600 mV/s. These results indicate that the $[4\text{-Fe}]^{2-}$ cluster exists in a lecithin layer formed on the surface of the HMDE, and only the cluster adsorbed on the Hg electrode undergoes a reversible one-electron redox reaction due to the $[4\text{-Fe}]^{2-/3-}$ couple. The number of clusters adsorbed on the Hg electrode, therefore, is calculated from the areas of both anodic and cathodic waves.[16] The ratio of the amount of charge consumed in the anodic wave to that in the cathodic one, however, is 0.85 at the sweep rate 200 mV/s. When all the $[4\text{-Fe}]^{2-}$ clusters adsorbed on the electrode were reduced to $[4\text{-Fe}]^{3-}$ by applying –0.80 V vs. SCE to the electrode for 1 min, followed by the potential sweep between –0.80 and –0.30 V, the area of the anodic wave is almost identical with that of the cathodic one as shown

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(11) The $E_{1/2}$ values used in this study are approximated as an average of cathodic and anodic peak potentials.
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(15) (a) Miller, I. R.; Bach, D. *J. Colloid Interface Sci.* **1969**, *29*, 250. (b) Müller, E.; Dörfler, H.-D. *Bioelectrochem. Bioenerg.* **1980**, *7*, 459.
(16) The peak current $i$ arising from a reversible couple of adsorbed species is expressed by $i = (n^2F^2/4RT)\,vA\Gamma$, where $n$, $F$, $v$, $A$, and $\Gamma$ are the number of electrons transfered, the Faraday constant, the scan rate, the area of an electrode, and the surface concentration of adsorbed species, respectively: Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; John Wiley & Sons: New York, 1980; p 522.

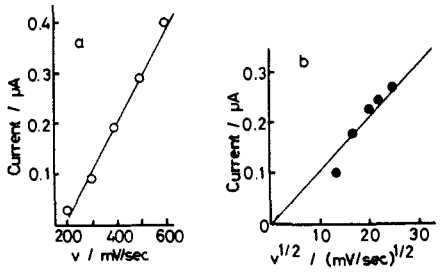


**Figure 4.** Plots of the anodic peak current at −0.645 V vs. the sweep rate (a) and that at −0.535 V vs. a square root of the sweep rate (b).

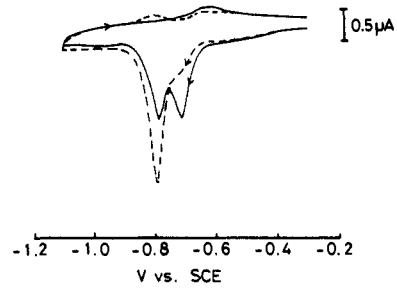


**Figure 5.** Cyclic voltammograms of $[4\text{-Fe}]^{2-}$ (10.6 μmol) solubilized in an aqueous lecithin vesicle solution (30 cm³) at pH 9.40 after $t_{exp}$ = 1 min (—) and $t_{exp}$ = 5 min (---).

in Figure 2b. Thus, the reduced species $[4\text{-Fe}]^{3-}$ is partly desorbed from the Hg electrode, and the adsorption of the oxidized species $[4\text{-Fe}]^{2-}$ is much slower than the cyclic voltammetry time scale.

The CV of $[4\text{-Fe}]^{2-}$ solubilized in lecithin vesicles at pH 6.72 being somewhat different from that at pH 5.65 shows one cathodic wave at −0.600 V vs. SCE and two anodic waves at −0.600 and −0.530 V vs. SCE at the sweep rate 200 mV/s after $t_{exp}$ = 1 min, as depicted in Figure 3a. The agreement in the potential between the cathodic wave and one of the anodic waves (−0.600 V) indicates that the redox reaction is associated with the cluster adsorbed on the Hg electrode. In fact, the peak currents of these two waves are proportional to the sweep rates in the range 200–600 mV/s, whereas the anodic wave at −0.530 V is gradually weakened with an increase in the sweep rate (Figure 3a). There may be a possibility that the anodic wave at −0.530 V results from the oxidation of octylbenzenethiolate liberated from $[4\text{-Fe}]^{3-}$ adsorbed on the Hg electrode. However, this is not the case, since the CV of octylbenzenethiol solubilized in lecithin vesicles has shown the anodic and cathodic waves at −0.640 and −0.645 V vs. SCE, respectively, at pH 6.70. In view of the fact that $[4\text{-Fe}]^{3-}$ has a tendency to desorb from the electrode, as described above, the −0.530 V anodic wave observed at pH 6.72 may, therefore, be associated with the oxidation of $[4\text{-Fe}]^{3-}$ desorbed from the electrode.

The desorption of $[4\text{-Fe}]^{3-}$ from the surface of the HMDE seems to occur more easily at pH 7.41, where a couple of cathodic and anodic waves at −0.645 and −0.535 V, respectively, are observed at the sweep rate 200 mV/s after $t_{exp}$ = 1 min, as shown in Figure 3b. At the sweep rate 300 mV/s, a new anodic wave due to a nearly reversible surface redox reaction appears at −0.645 V as a shoulder, and is strengthened with an increase in the sweep rates (Figure 3b). The peak currents of the −0.645 and −0.535 V anodic waves are proportional to first order and one-half order, respectively, with respect to the sweep rate in the range 200–600 mV/s, as shown in Figure 4, which suggests that the latter does not arise from an adsorbed species.[16] It may, therefore, be concluded that the −0.535 V anodic wave results from a normal electron transfer between $[4\text{-Fe}]^{3-}$ dissolved in (not adsorbed on) a lecithin layer and the electrode. In accordance with this, when all $[4\text{-Fe}]^{2-}$ adsorbed on the HMDE were reduced to $[4\text{-Fe}]^{3-}$ at −0.80 V for 1 min, followed by the potential sweep between −0.80 and −0.30 V, the −0.645 V anodic wave completely disappeared even at the sweep rate 600 mV/s. Thus, in the neutral region, most of the $[4\text{-Fe}]^{3-}$ is subject to desorption from the surface of the HMDE to diffuse in the lecithin layer on the electrode, undergoing the oxidation reaction at −0.535 V.

The CV of $[4\text{-Fe}]^{2-}$ solubilized in lecithin vesicles at pH 9.40 exhibits two cathodic (−0.704 and −0.792 V) and one anodic (−0.620 V) waves at the sweep rate 200 mV/s after $t_{exp}$ = 1 min, as shown by a solid line in Figure 5. An extension of $t_{exp}$ to 5 min results in the strengthening of the −0.792 V cathodic wave and the appearance of a new anodic wave at −0.792 V at the same sweep rate, while the −0.704 V cathodic wave is extremely weakened (a broaken line in Figure 5). On the basis of the preceding discussion, a pair of cathodic and anodic waves at −0.792 V are attributable to the redox couple due to the cluster adsorbed on the HMDE. On the other hand, when the potential of the HMDE had been maintained at −1.00 V for 2 min, followed by the potential sweep between −1.00 and −0.30 V, the redox couple at −0.792 V disappeared, while the −0.704 cathodic and the −0.620 V anodic waves are strengthened. It should be mentioned here that there has been observed neither cathodic nor anodic waves arising from octylbenzenethiol solubilized in lecithin vesicles, which exhibited those waves at −0.840 and −0.835 V, respectively, at the sweep rate 200 mV/s at pH 9.40. This result indicates that $[4\text{-Fe}]^{2-}$ may not be decomposed upon the redox reaction in an alkaline solution, whereas *Clostridium pasteurianum* ferredoxin adsorbed on an HMDE is decomposed upon the reduction in alkaline solutions and the resulting apoprotein is strongly adsorbed on the HMDE with sulfur atoms of the cysteine residues.[17] Thus, if one can assume that the rate of the adsorption of $[4\text{-Fe}]^{2-}$ on the HMDE in alkaline solutions is slower than that in acidic solutions, the −0.704 V cathodic and the −0.620 V anodic waves are assignable to a normal electron transfer between the cluster dissolved in a lecithin layer and the electrode.

**The Rate of Migration of $[4\text{-Fe}]^{2-}$ in a Lecithin Layer.** As mentioned in the previous section, the CV of $[4\text{-Fe}]^{2-}$ solubilized in lecithin vesicles in acidic media shows only a redox couple due to the adsorbed species. This is in contrast to that in alkaline media, where the $[4\text{-Fe}]^{2-}$ species not only adsorbed on the HMDE but also dissolved in a lecithin layer undergo the redox reaction at the electrode. The thickness of the lecithin layer formed on the HMDE may be much larger than that of an electrical double layer since the lecithin layer is composed of 15 molecular layers at least (vide infra). Thus, in acidic media, $[4\text{-Fe}]^{2-}$ dissolved in the lecithin layer slowly migrates into an electrical double layer of the Hg electrode, followed by fast adsorption to the electrode surface. The electron-transfer reaction between the $[4\text{-Fe}]^{2-}$ cluster and the electrode may, therefore, be controlled by diffusion of the cluster in the lecithin layer.

The diffusion current ($i_t$) detected on a spherical electrode with the radius $r$ at the time $t$ is expressed by eq 3,[18]

$$i_t = nFAC^*D[(\pi Dt)^{-1/2} + r^{-1}] \quad (3)$$

where $n$, $F$, $A$, $C^*$, and $D$ are the number of electrons transfered, the Faraday constant, the surface area of the electrode, the concentration of the electroactive species, and diffusion coefficient of the electroactive species, respectively. Integration of eq 3 by $t$ from 0 to $t_{exp}$ affords eq 4 for the amount of charge $Q$ consumed on the electrode in the time $t_{exp}$. In the present study, the $Q$ value

$$Q = nFAC^*[2D^{1/2}\pi^{-1/2}t_{exp}^{1/2} + Dr^{-1}t_{exp}] \quad (4)$$

may be identical with the amount of charge consumed in the cathodic wave of the $[4\text{-Fe}]^{2-/3-}$ couple in acidic media, since there occurs only the redox reaction of adsorbed clusters. The amount of charge consumed in the cathodic wave is expected to depend not only on the concentration of the cluster but also on the time of exposure ($t_{exp}$). In fact, the area of the cathodic wave at pH 5.65 increases with increasing $t_{exp}$, as shown in Figure 6. Plots of the square of the amount of charge consumed in the cathodic wave of $[4\text{-Fe}]^{2-}$ at pH 5.65 against $t_{exp}$ are depicted in Figure

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(18) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods*; John Wieley & Sons: New York, 1980; p 145.

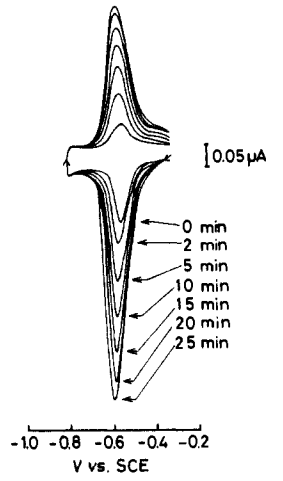


**Figure 6.** Cyclic voltammograms of $[4\text{-Fe}]^{2-}$ (3.2 μmol) solubilized in an aqueous lecithin vesicle solution (30 $cm^3$) at pH 5.65 after various $t_{exp}$; sweep rate 200 mV/s.

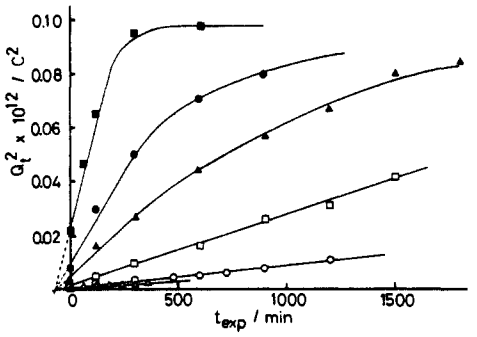


**Figure 7.** Plots of $Q^2$ vs. $t_{exp}$ under various $C_{app}$ values in $H_2O$ at pH 5.65; $C_{app}$ = 2.08 × $10^{-4}$ (■), 1.56 × $10^{-4}$ (●), 1.04 × $10^{-4}$ (▲), 6.24 × $10^{-5}$ (□), 3.12 × $10^{-5}$ (○), and 2.49 × $10^{-5}$ (△) mol/$dm^3$.

7, which shows a linear relation between those two values at various concentrations $C_{app}$ calculated from the amount of $[4\text{-Fe}]^{2-}$ added in 30 $cm^3$ of water until the square of the amount of charge comes to a certain value in each concentration of $[4\text{-Fe}]^{2-}$. This result suggests that the diffusion coefficient $D$ of $[4\text{-Fe}]^{2-}$ in a lecithin layer is negligibly small compared with the value of $r$ in eq 4. Therefore, eq 4 is approximated by eq 5, which is known as the

$$Q = 2nFAC^*(D/\pi)^{1/2}(t_{exp})^{1/2} \quad (5)$$

equation for the rate of adsorption of electroactive species onto a planar electrode.[19] The presence of non-zero intercepts at each plot (Figure 7) reveals that a given amount of $[4\text{-Fe}]^{2-}$ is already adsorbed on the Hg electrode even in the time required to adjust the surface area of the HMDE to 0.0187 $cm^2$. In fact, on extraporation of the linear parts to zero of $Q^2$, all lines come together around −60 s (broaken lines in Figure 7). The $C^*D^{1/2}$ value of eq 5 can be obtained from the slope of a line at each $C_{app}$ value (Figure 7). However, the direct determination of the $C^*$ value is difficult, since not only the value in the present study is the concentration of $[4\text{-Fe}]^{2-}$ in a lecithin layer formed on the HMDE but also only a part of $[4\text{-Fe}]^{2-}$ dissolved in a lecithin layer is detected as an adsorbed species in the cyclic voltammogram.

For the ordinary size of a single-layer vesicle with the diameter of about 250 Å and the membrane thickness of 43 ± 3 Å, the aggregation number of lecithin has been reported as 4000.[20,21] The volume occupied by one molecule of lecithin in a single-layer vesicle, therefore, is estimated as 1500 ± 100 $Å^3$. When the volume of lecithin is assumed to be no different between single-

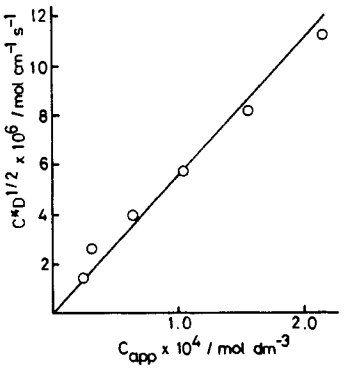


**Figure 8.** A plot of $C^*D^{1/2}$ vs. $C_{app}$.

and multi-layer vesicles, the total volume of the membrane in vesicles in the present study may be 0.11 ± 0.01 $cm^3$, based on the concentration of lecithin in water (4.0 × $10^{-3}$ mol/$dm^3$, 30 $cm^3$). The $[4\text{-Fe}]^{2-}$ cluster added to a vesicle solution is solubilized in the bilayer membrane. The $C^*$ value can, therefore, be related with $C_{app}$ as $C^* = (270 \pm 20)C_{app}$, suggesting that $[4\text{-Fe}]^{2-}$ is concentrated by about 270 times in the lecithin vesicle, compared with the homogeneous solution of $[4\text{-Fe}]^{2-}$. The concentration of $[4\text{-Fe}]^{2-}$ in lecithin vesicles may be maintained also in the lecithin layer on the HMDE, since the $C^*D^{1/2}$ values obtained from the slopes of each line in Figure 7 show a linear relation with the corresponding $C_{app}$ values as depicted in Figure 8, which clearly indicates that $[4\text{-Fe}]^{2-}$ is distributed homogeneously into the lecithin layer on the HMDE. The diffusion coefficient $D$ of $[4\text{-Fe}]^{2-}$ in the lecithin layer, therefore, is determined as (8.7 ± 1.1) × $10^{-9}$ $cm^2/s$. This value is much smaller than that of a single-layer vesicle of lecithin in $H_2O$ ($D$ = (1.87–2.35) × $10^{-7}$ $cm^2/s$),[9,22] suggesting a restricted movement of $[4\text{-Fe}]^{2-}$ in the lecithin membrane. It is, however, noteworthy that the present $D$ value is very close to a charge transport diffusion coefficient ($D_{CT}$ = 1 × ($10^{-10}$–$10^{-9}$) $cm^2/s$) of an electron self-exchange between neighboring oxidized and reduced sites of transition-metal ions such as $Fe^{2+/3+}$, $Ru^{2+/3+}$, and $Os^{2+/3+}$ affixed in electrochemically polymerized thin-layer vinylpyridine, -bipyridine, and -phenanthroline derivatives on electrodes.[23]

The saturation of the $Q^2$ value at the high $C_{app}$ values (■ and ● in Figure 7) indicates that the surface of the HMDE is covered almost completely by the monolayered $[4\text{-Fe}]^{2-}$ cluster. The maximum concentration of $[4\text{-Fe}]^{2-}$ adsorbed on the electrode is estimated as 1.75 × $10^{-10}$ mol/$cm^2$ at pH 5.65. This value also provides information regarding the thickness of the lecithin layer on the HMDE, since the concentration of $[4\text{-Fe}]^{2-}$ in lecithin vesicles may be maintained in the lecithin layer formed on the HMDE, as discussed above. The number of molecules on the outer and the inner surface of a bilayer membrane of a lecithin vesicle with an aggregation number 4000 is 2800 and 1200, respectively,[24] suggesting that the area occupied by one molecule of lecithin on the outer surface of the vesicles is 70 $Å^2$/molecule. In addition, the cross-sectional area of the paraffinic chains of lecithin adsorbed on an Hg electrode is estimated also as 70 $Å^2$/molecule.[15a] Therefore, the 2.58 × $10^{12}$ lecithin molecules may be adsorbed directly on the surface of the HMDE (0.018 $cm^2$) as a monomolecular layer. However, the maximum number of $[4\text{-Fe}]^{2-}$ which may be involved in such a lecithin monomolecular layer is 1.34 × $10^{11}$ under the present experimental conditions (■ in Figure 7). The discrepancy of the number of $[4\text{-Fe}]^{2-}$ adsorbed on the HMDE between the observed (1.95 × $10^{12}$) and the calculated values (1.34 × $10^{11}$) may be explained by a formation of

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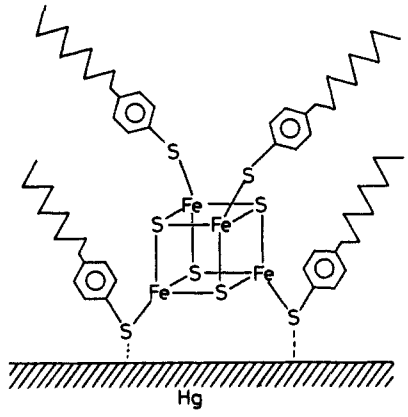


**Figure 9.** The proposed structure of $[4\text{-Fe}]^{2-}$ adsorbed on an Hg electrode.

a lecithin multimolecular layer (like a lamella) on the HMDE rather than a monomolecular one, suggesting that the lecithin layer formed on the electrode is composed of 7–8 bilayer membranes at least.

It is well-known that not only organic but also inorganic sulfur compounds are strongly adsorbed on the surface of an Hg electrode.[25] In accordance with this, the redox couple of $[4\text{-Fe}]^{2-}$ solubilized in lecithin vesicles has not been observed at all even in acidic conditions when a glassy carbon electrode was used in place of an HMDE. In view of steric requirement, the $[4\text{-Fe}]^{2-}$ cluster may be bound to the HMDE with two terminal sulfur atoms, as illustrated in Figure 9, rather than bridging sulfur atoms. When $[4\text{-Fe}]^{2-}$ adsorbed on an HMDE is assumed to adopt a similar structure to $[Fe_4S_4(SC_6H_5)_4]^{2-}$ in the solid state, the maximum surface concentration of $[4\text{-Fe}]^{2-}$ is estimated as $1.60 \times 10^{-10}$ mol/cm$^2$, which is essentially consistent with the observed value at pH 5.65. This result indicates that the surface of the HMDE is covered almost completely by a monomolecular layered $[4\text{-Fe}]^{2-}$. Most of the lecithin molecules which were adsorbed directly on the HMDE may, therefore, be replaced by the clusters. The surface concentration of *Clostridium pasteurianum* ferredoxin adsorbed on an Hg electrode is $1.28 \times 10^{-11}$ mol/cm$^2$, which is smaller than that of $[4\text{-Fe}]^{2-}$. This may be explained by the bulkiness of the peptide chain of the ferredoxin.

**The $E_{1/2}$ Value of the $[4\text{-Fe}]^{2-/3-}$ Couple at Various pH.** Figure 10 shows plots of the $E_{1/2}$ values[11] of the clusters not only adsorbed on the surface of the HMDE but also dissolved in a lecithin layer against pH value. Each plot exhibits a linear relation with the slope −55 mV/pH in the pH range lower than 10.5, indicating that a single proton participates in the redox reaction of the cluster.[26] Similar pH dependence of $E_{1/2}$ has been reported not only for some iron–sulfur proteins of mitochondria site I[27] but also for $[Fe_4S_4(SCH_2CH_2COO^-)_4]^{6-}$ dissolved in an aqueous alubmin solution,[28] both of which have exhibited a slope −60 mV/pH in the pH range lower than 8.0 at ambient temperature. In the present study, the $[4\text{-Fe}]^{2-}$ cluster solubilized in a lecithin membrane may exist as a deprotonated species in the pH range higher than 10.5, resulting in the redox potential being almost constant. Even in that pH region, the $E_{1/2}$ value of $[4\text{-Fe}]^{2-}$ dissolved in a lecithin layer is still 380 mV more positive than that in anhydrous $Me_2SO$. The potential difference of $E_{1/2}$ and $Me_2SO$ may, therefore, be explained by any solvent effects other than protonation, though the details have not been clarified in the present study.

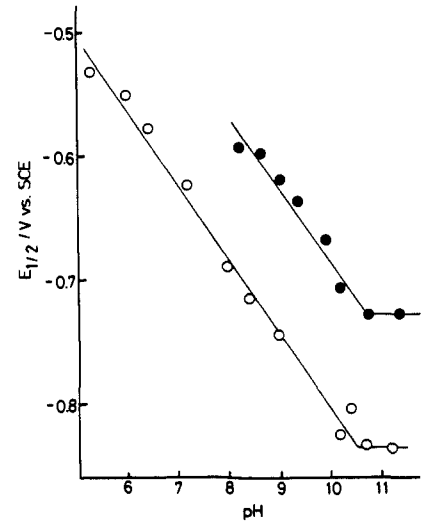


**Figure 10.** Plots of the $E_{1/2}$ of the cluster adsorbed on the surface of the HMDE (○) and dissolved in a lecithin layer (●) vs. pH.

Although the $E_{1/2}$ values of the clusters adsorbed on the HMDE surface and dissolved in a lecithin layer at each pH (Figure 10) are different by 80 mV from each other, the p$K$ values determined from each break point of the relations between $E_{1/2}$ and pH are both 10.5, which is somewhat larger than those of iron–sulfur proteins reported previously (p$K$ = 6.5–8.9).[29] The pH dependence of the adsorption behavior of $[4\text{-Fe}]^{2-}$ on the HMDE may be explained by the equilibrium between the protonated and deprotonated clusters: in alkaline solutions, most of $[4\text{-Fe}]^{2-}$ exist as deprotonated species. An accumulation of the deprotonated cluster on the electrode may induce a repulsion between the neighboring clusters to each other owing to their negative charge. On the other hand, $[4\text{-Fe}]^{2-}$ undergoes a protonation reaction in acidic solutions, resulting in a decrease of a formal charge of the cluster. A coulomb repulsion between the clusters in acidic conditions may, therefore, be weaker than that in alkaline ones. Thus, the adsorption of $[4\text{-Fe}]^{2-}$ on the HMDE is more prominent in acidic media than in alkaline media.

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(26) When a single proton is involved in the redox reaction of $[4\text{-Fe}]^{2-}$, $E_{1/2}$ can be expressed by

$$E^{\circ\prime} + \frac{RT}{F} \ln \frac{[4\text{-Fe}(H^+)]^{2-}}{[4\text{-Fe}]^{3-}} - \frac{2.303RT}{F}(\text{pH} - \text{p}K)$$

where $E^{\circ\prime}$ and $[4\text{-Fe}(H^+)]^{2-}$ are the formal potential of an electrode and the protonated cluster, respectively.[7] Thus, the slope of the plot of $E_{1/2}$ vs. pH is −55 mV/pH at 4 °C.

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