  Bull. Chem. Soc. Jpn., 61, 1285—1289 (1988)

Electron Coupled Proton Transport Mediated by $[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]^{2-}$ in Liquid Membrane

Koji TANAKA,* Mari MASANAGA, and Toshio TANAKA*
Department of Applied Chemistry, Faculty of Engineering, Osaka University, Yamada-Oka, Suita, Osaka 565
(Received October 28, 1987)

Electrochemical reduction of $[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]^{2-}$ ($[4\text{-Fe}]^{2-}$) dissolved in a CH_2Cl_2 phase of the CH_2Cl_2/H_2O two phase system is followed by protonation to give $[4\text{-Fe}]^{3-}\text{-}H^+$ when the pH of the H_2O phase is lower than 7.0, and protonated and deprotonated $[4\text{-Fe}]^{3-}$ exist as an equilibrium mixture in the CH_2Cl_2 phase. On the other hand, no protonation takes place upon the reduction of $[4\text{-Fe}]^{2-}$ in the CH_2Cl_2 phase contacting with the H_2O phase with the pH higher than 7.0. The electron transport conducted in a liquid membrane composed of the H_2O (W_1), CH_2Cl_2, and H_2O (W_2) phases containing sodium dithionite as an electron donor, $(n\text{-}Bu_4N)_2[4\text{-Fe}]$ as a mediator, and disodium anthraquinone-1,5-disulfonate (Na_2AQS) as an electron acceptor, respectively, revealed that protonated $[4\text{-Fe}]^{3-}\text{-}H^+$ mediates electron coupled proton transport in the liquid membrane, while the deprotonated cluster has no ability of electron transport in the same system.

Electron transport across a cell membrane plays key roles not only in various biological redox reactions but also in ATP synthesis in mitochondria or chloroplasts. Model studies for the electron transport have, therefore, widely been conducted by using bilayer lipid membranes[1-5] or liquid membranes.[6-9] Electron transport in biological membranes takes place according to an electrochemical proton gradient, where electrons and protons are transported from the high proton concentration side to the low proton concentration side across a biomembrane.[10-14] A pH dependence of the redox potential of an oxidation-reduction component in a biomembrane provides evidence for a proton participation in the redox reaction. Mitochondrial cytochromes which show the shift of −60 mV/pH for their half-reduction potentials have, therefore, been considered to take part in electron coupled-proton transport.[15-17] The intermediary which plays in such a role at negative potentials more than the redox reactions of cytochromes, however, has not been elucidated so far.

Iron-sulfur proteins widely distributed in plants, bacteria, and mammals are considered soley to function as electron transfer catalysts in various biological redox reactions such as nitrate reduction, CO_2- and N_2-fixation, H_2-evolution, and so on.[18] In accordance with this, the redox potentials of most Fe-S proteins show a relatively small shift (0 to −24 mV/pH) with the change of pH,[19-21] while that of the mitochondrial Fe-S Center N-2 shows a slope of −60 mV/pH,[22,23] implying a stoichiometric one electron and one proton participation in the redox reaction. Mitochondrial Fe-S Center N-2, therefore, has been proposed to function not only as electron but also as proton carriers in biomembranes.[22]

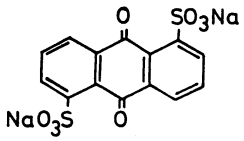
The interactions between synthetic Fe_4S_4 clusters and protons have also been studied; the pK_a values determined spectrophotometrically[24,25] and electrochemically[26-30] fall in the range 3.9 to 9.0. We have, recently, elucidated that reduction-linked proton binding to $[Fe_4X_4(YC_{12}H_{25})_4]^{3-}$ (X, Y=S and Se) takes place at the sulfur or selenium atom of the Fe_4X_4 core rather than that of terminal alkanethiolate and -selenolate ligands on the basis of their pK_a values in aqueous Triton X-100 micellar solutions.[31] The ability of proton binding to the Fe_4S_4 cluster solubilized in hydrophobic spheres in aqueous micellar solutions suggests that the synthetic Fe_4S_4 cluster can be used as a mediator of the electron coupled proton transport in a liquid membrane. In fact, $[Fe_4S_4(SPh)_4]^{3-}$ prepared by reduction of $[Fe_4S_4(SPh)_4]^{2-}$ with Cr^{II} in a toluene/water two phase system is protonated and releases the proton upon reoxidation with methylviologen.[32] This paper describes electron coupled proton transport from aqueous sodium dithionite ($Na_2S_2O_4$) to disodium anthraquinone-1,5-disulfonate solutions across a CH_2Cl_2 phase containing $[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]^{2-}$.

Experimental

General. All manipulations were carried out under an N_2 atmosphere. The iron-sulfur cluster $(n\text{-}Bu_4N)_2[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]$ ($(n\text{-}Bu_4N)_2[4\text{-Fe}]$) was prepared according to the literature.[28]

Physical Measurements. Cyclic voltammograms were obtained by the use of a Hokuto Denko potentialstat HB-401, a Hokuto Denko function generator HB-107, and a Yokogawa Electric X-Y recorder 3077. The electrolysis cell was equipped with a nozzle for bubbling N_2. The concentrations of Na^+ in aqueous solutions were determined by the use of a Nippon Jarrell-Ash atomic absorption spectrophotometer AA-8500.

Cyclic Voltammetry of $[4\text{-Fe}]^{2-}$ in an H_2O/CH_2Cl_2 Two Phase System. An aqueous H_3PO_4-NaOH buffer (0.1 mol dm^{-3}) solution (pH 9.4 or 6.5, 20 cm^3) containing $n\text{-}Bu_4NBr$ (0.6 g, 1.8 mmol) was poured in a CH_2Cl_2 solution (20 cm^3) containing $(n\text{-}Bu_4N)_2[4\text{-Fe}]$ (20 mg, 12 μmol) and $n\text{-}Bu_4NBr$ (0.6 g, 1.8 mmol), and the mixture was stirred by bubbling N_2 for several minutes. Then, the cell was allowed to stand until the CH_2Cl_2 and H_2O phases were separated completely. The proton concentration in the H_2O phase was adjusted by addition of a small amount of either aqueous NaOH or H_3PO_4 solution to the H_2O phase, followed by



AQS^{2-} : $E_{1/2}$ = -0.43 V (pH 7.0)

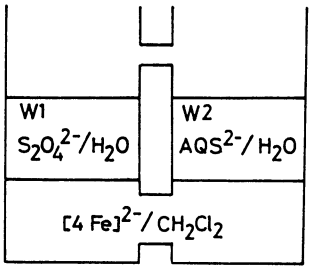


Fig. 1. A liquid membrane cell.

stirring the mixture by the same method. The pH of the H_2O phase was measured with a Toa Electronics pH meter HM-7B. The cyclic voltammogram of $(n\text{-}Bu_4N)_2$[4-Fe] solubilized in the CH_2Cl_2 phase was obtained by using a Yanaco glassy carbon disk electrode GC-2P, a Pt auxiliary electrode, and a luggin capillary of a reference electrode (SCE), all of which were immersed into the CH_2Cl_2 phase.

Electron Coupled Proton Transport across a Liqid Membrane. The electron coupled proton transport across a liquid membrane, mediated with $(n\text{-}Bu_4N)_2$[4-Fe] was conducted by using a liquid membrane cell (16 mm inner diameter) (Fig. 1). After the cell was thoroughly flushed with N_2 to remove air, a CH_2Cl_2 solution (20 cm^2) of $(n\text{-}Bu_4N)_2$[4-Fe] (2.5—20 mg, 1.5—12 μmol) was charged into the cell with syringe techniques through a septum cap attached to the top of the cell. Then, aqueous H_3PO_4-NaOH buffer solutions (0.1 mol dm^{-3}, 5 cm^3) of $Na_2S_2O_4$ (0.108 g, 0.62 mmol) and of disodium anthraquinone-1,5-disulfonate (Na_2AQS) (4.9—25 mg, 0.12—0.61 mmol) were introduced into the cell as an electron donor (W_1 phase) and an electron acceptor (W_2 phase), respectively, and the CH_2Cl_2 layer was stirred magnetically at 20 °C for 16 h in the dark owing to instability of Na_2AQS toward light. Electron transport from the W_1 to the W_2 phases through the CH_2Cl_2 phase was monitored by the change of an absorption band centered around 450 nm due to the reduced species of AQS^{2-} formed in the W_2 phase, and the electrons transported from the W_1 phase to the W_2 phase were determined from the absorbance at 450 nm. In the present study, the 450 nm band has been assigned to a two-electron reducticon product of AQS^{2-}, though several reduced species of AQS^{2-} exist as an equilibrium mixture in aqueous solutions.[33] The apparent amount of the reduced species of AQS^{2-} was calculated from the absorbance at 450 nm upon dissolving a given amount of $Na_2S_2O_4$ (as a two electron donor) in aqueous Na_2AQS solutions with the same concentration as the W_2 phase at various pH.

Results and Discussion

The Redox Behavior of $(n\text{-}Bu_4N)_2[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]$ in an H_2O/CH_2Cl_2 Two Phase System. As reported previously, redox potentials of the $[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]^{2-/3-}$ ([4-Fe]$^{2-/3-}$) couple in aqueous micellar[27,28,30] and lecithin vesicle solutions[29] are largely influenced by proton concentrations owing to the redox-linked protonation of the cluster. Thus, the redox behavior of the same cluster dissolved in the CH_2Cl_2 phase of a CH_2Cl_2/H_2O two phase system was examined with the intention of applying to an electron coupled proton transfer catalyst in a liquid membrane. The cyclic voltammogram of $(n\text{-}Bu_4N)_2$[4-Fe] in a CH_2Cl_2/H_2O (pH 9.40) two phase system shows a pair of cathodic and anodic waves due to the [4-Fe]$^{2-/3-}$ redox couple at −0.99 and −0.85 V vs. SCE, respectively, at the sweep rate 10 mV s^{-1} (Fig. 2). The cathodic and anodic peak potentials are moved in dif-

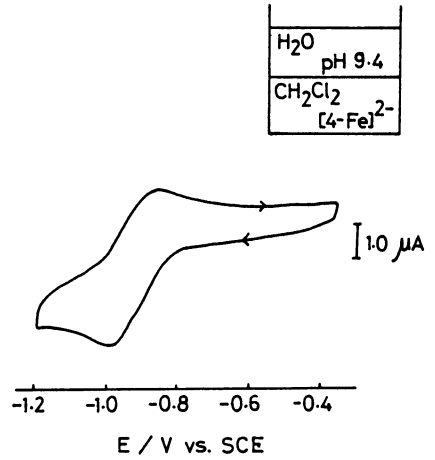


Fig. 2. Cyclic voltammogram of $(n\text{-}Bu_4N)_2[Fe_4S_4(SC_6H_4\text{-}p\text{-}n\text{-}C_8H_{17})_4]$ solubilized in the CH_2Cl_2 layer (0.60 mmol dm^{-3}) in the H_2O/CH_2Cl_2 two phase system; dE/dt=10 mV s^{-1}.

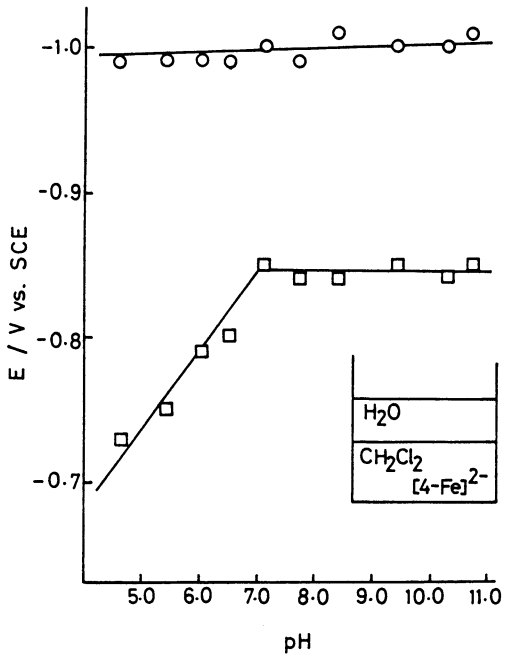


Fig. 3. Plots of the cathodic (○) and anodic peak potentials (□) of the [4-Fe]$^{2-/3-}$ redox couple in the H_2O/CH_2Cl_2 system vs. pH of the H_2O phase.

ferent fashions from each other upon change of the proton concentration in the H_2O phase (Fig. 3); the cathodic peak potential is almost independent of pH of the H_2O phase in the pH range 4.6 to 11.0 (circles in Fig. 3), suggesting that no proton transport is involved in the reduction of $[4\text{-Fe}]^{2-}$ in the H_2O-saturated CH_2Cl_2 phase. On the other hand, the anodic peak potential of the redox couple varies with a slope −55 mV/pH in the pH range 4.6 to 7.0, while it is independent of pH in the range higher than 7.0 (squares in Fig. 3). Such characteristic pH dependences of the cathodic and anodic peak potentials of the $[4\text{-Fe}]^{2-/3-}$ couple in the H_2O-saturated CH_2Cl_2 layer may be explained by assuming Eqs. 1—4. The cluster in the

$$[4\text{-Fe}]^{2-} + e^- \longrightarrow [4\text{-Fe}]^{3-} \quad (1)$$

$$[4\text{-Fe}]^{3-} + H^+ \overset{pH<7}{\rightleftharpoons} [4\text{-Fe}]^{3-}\text{-}H^+ \quad (2)$$

$$[4\text{-Fe}]^{3-} - H^+ \overset{pH<7}{\longrightarrow} [4\text{-Fe}]^{2-} + e^- + H^+ \quad (3)$$

$$[4\text{-Fe}]^{3-} \overset{pH>7}{\longrightarrow} [4\text{-Fe}]^{2-} + e^- \quad (4)$$

CH_2Cl_2 phase undergoes one-electron reduction to afford $[4\text{-Fe}]^{3-}$ (Eq. 1), followed by protonation, probably at the sulfur atom of the Fe_4S_4 core[31] in the CH_2Cl_2 phase when pH of the H_2O phase is lower than 7, where protonated and deprotonated clusters exist as an equilibrium mixture (Eq. 2). Upon subsequent oxidation, the protonated cluster $[4\text{-Fe}]^{3-}\text{-}H^+$ may release the proton owing to decreasing electron density of the Fe_4S_4 core (Eq. 3). On the other hand, the cluster may be subject to the redox reaction without undergoing protonation (Eqs. 1 and 4) in the pH range of the H_2O phase higher than 7.0, explaining the anodic peak potential being almost invariant in that pH range (squares in Fig. 3). The pK_a value 7.0 of $[4\text{-Fe}]^{3-}\text{-}H^+$ obtained from the turning point of the anodic peak potential[34] (Fig. 3) is smaller than that in aqueous micellar solutions (pK_a 9.0).[27,28] This result may partly be due to low proton concentrations in the H_2O-saturated CH_2Cl_2 phase compared with those in hydrophobic spheres in micells.

Electron Coupled Proton Transport across a Liquid Membrane Mediated with (n-Bu_4N)$_2$[4-Fe]. A proton binding to $[4\text{-Fe}]^{3-}$ in the CH_2Cl_2/H_2O two phase system suggests that the present cluster has an ability of the electron coupled proton transport. Thus, the electron transport mediated with the cluster was conducted by using a liquid membrane (Fig. 1); aqueous buffer solutions (H_3PO_4-NaOH) of sodium dithionite (W_1 phase) and of disodium anthraquinone-1,5-disulfonate (Na_2AQS) (W_2 phase) were used as an electron donor and an electron acceptor, respectively, on the CH_2Cl_2 phase containing (n-Bu_4N)$_2$[4-Fe]. The pH values of the W_1 (pH_1) and W_2 (pH_2) phases were adjusted at 6.2 and 10.7, respectively. Electron transport conducted by stirring the CH_2Cl_2 phase results in the occurrence of a strong absorption band centered around 450 nm due to the reduced species of AQS^{2-} in the electronic spectrum of the W_2 phase (a solid line in Fig. 4). On the other hand, no absorption band appears around 450 nm in the absence of (n-Bu_4N)$_2$-[4-Fe] in the CH_2Cl_2 phase under otherwise the same conditions (a broken line in Fig. 4). Thus, the iron-sulfur cluster in the CH_2Cl_2 phase mediates electron coupled H^+ or Na^+ transport from the W_1 to the W_2 phases, since no other counter cation is involved in this system. In view of the fact that protonation to $[4\text{-Fe}]^{3-}$ takes place only in the pH region lower than 7.0 of the H_2O phase in the CH_2Cl_2/H_2O two phase system, the electron coupled proton transport mediated

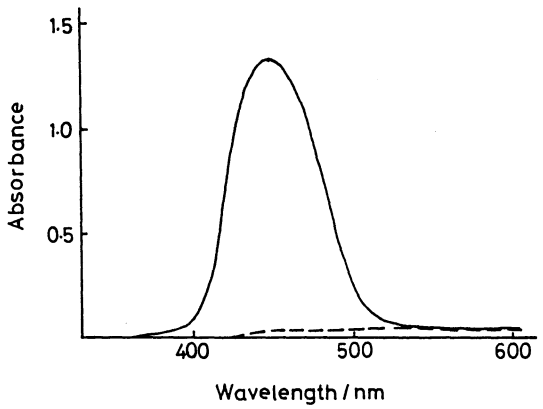


Fig. 4. Electronic absorption spectra of the reduced species of AQS^{2-} formed in the W_2 phase in the absence (----) and the presence of (n-Bu_4N)$_2$[4-Fe] (10 μmol) (——) in the CH_2Cl_2 (20 cm^3) phase after electron transport for 16 h; the concentrations of both $Na_2S_2O_4$ in the W_1 (H_2O, 5 cm^3; pH 6.2) and Na_2AQS in the W_2 (H_2O, 5 cm^3; pH 10.7) phases are 1.2×10^{-1} mol dm^{-3}.

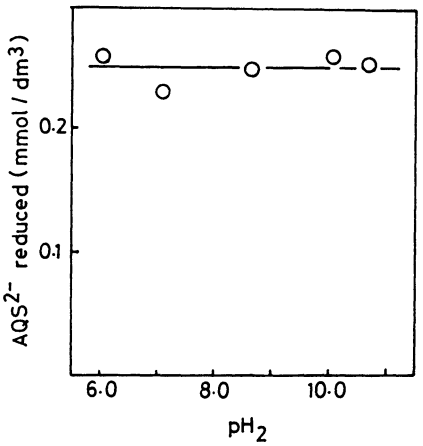


Fig. 5. Amounts of the reduced species of AQS^{2-} formed in the electron transport mediated with $[4\text{-Fe}]^{2-}$ at various pH of the W_2 phase for 16 h; the concentrations of $Na_2S_2O_4$ in the W_1 (H_2O, 5 cm^3; pH 6.2), (n-Bu_4N)$_2$[4-Fe] in the CH_2Cl_2 (20 cm^3), and Na_2AQS in the W_2 (H_2O, 5 cm^3) phases are 1.2×10^{-1}, 1.5×10^{-4}, and 1.2×10^{-1} mol dm^{-3}, respectively.

with $[4\text{-Fe}]^{3-}$ across the CH_2Cl_2 phase may largely be influenced by proton concentrations of the H_2O phase.

The amounts of the reduced species of AQS^{2-} formed in the W_2 phase is essentially constant irrespective of pH of this phase (pH) as far as pH of the W_1 phase (pH_1) is kept to be lower than 7; Fig. 5 is an example at pH_1 6.2. Thus, pH of the W_2 phase (pH_2) little influences the electron transfer from the reduced species of the cluster to AQS^{2-} at the CH_2Cl_2/W_2 interface. On the other hand, pH_1 largely influences on the formation of the reduced species of AQS^{2-} in the W_2 phase with pH_2 10.7; the amount of the reduced species of AQS^{2-} is rapidly decreased with increasing the pH value of the W_1 phase (Fig. 6). The agreement of the turning point (pH_1 7.0) in Fig. 6 with the pK_a value of $[4\text{-Fe}]^{3-}$-H^+ obtained from the plot of the anodic peak potential of the cluster vs. pH in the CH_2Cl_2/H_2O two phase system (Fig. 3) suggests that $[4\text{-Fe}]^{3-}$-H^+ rather than deprotonated cluster $[4\text{-Fe}]^{3-}$ mediates the electron transport from the W_1 phase to the W_2 phase. As an electron carrier, $[4\text{-Fe}]^{3-}$-H and $[4\text{-Fe}]^{3-}$ may exhibit a fairly different behavior from each other, since the former may have an ability of the electron coupled proton transport, while the latter mediates electron coupled Na^+ transport. The active species, $[4\text{-Fe}]^{3-}$-H^+ or $[4\text{-Fe}]^{3-}$, which mediates the electron transport from the W_1 to W_2 phases may, therefore, be assigned by determining the amount of Na^+ transported into the W_2 phase accompanied by the electron transport. The electron transport conducted by using $NaOH$-H_3PO_4 and KOH-H_3PO_4 buffer solutions in the W_1 and the W_2 phases, respectively, also produced

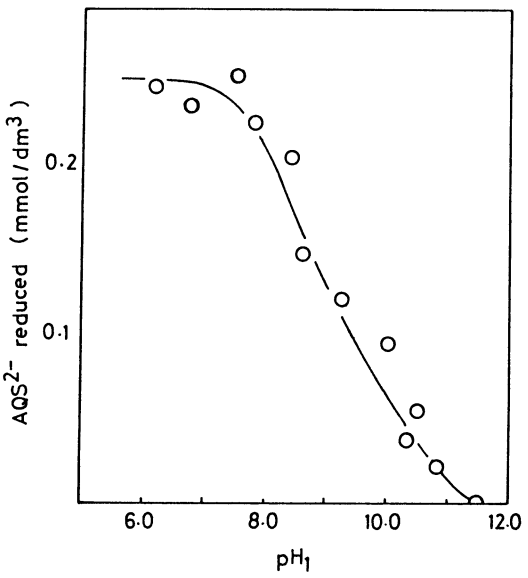
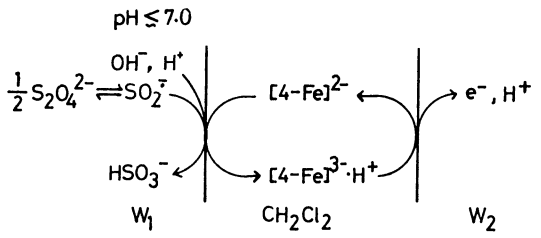


Fig. 6. The amount of the reduced species of AQS^{2-} formed in the electron transport mediated with $[4\text{-Fe}]^{2-}$ at various pH_1 (W_1 phase) for 16 h; the concentrations of $Na_2S_2O_4$ in the W_1 (H_2O, 5 cm^3), $(n\text{-}Bu_4N)_2[4\text{-Fe}]$ in the CH_2Cl_2 (20 cm^3), and Na_2AQS in the W_2 (H_2O, 5 cm^3; pH 10.7) phases are 1.2×10^{-1}, 1.5×10^{-4}, and 1.2×10^{-1} mol dm^{-3}, respectively.



Scheme 1.

the same amounts of the reduced species of AQS^{2-} in the W_2 phase as those in Fig. 6.[35] In addition, no appreciable change of the amount of Na^+ in the W_2 phase (resulting from Na_2AQS) was observed between before and after the electron transport. It may, therefore, be concluded that no appreciable Na^+ transport from the W_1 phase to the W_2 phase takes place in the present study. Thus $[4\text{-Fe}]^{3-}$-H^+ functions as an electron carrier in the liquid membrane. The maximum electrons transported in the present study was 2.0 on the basis of the amount of $(n\text{-}Bu_4N)_2[4\text{-Fe}]$ in the CH_2Cl_2 phase for 16 h.[36] Such a low value may be resulted from an endothermic electron transfer from $S_2O_4^{2-}$ (E=−0.90 V vs. SCE in H_2O at pH 9.0)[37] to $[4\text{-Fe}]^{2-}$ (E_{red}=−0.99 V in CH_2Cl_2) at the W_1/CH_2Cl_2 interface.

Electron Pathway in the Liquid Membrance. A most possible electron pathway for the present electron coupled proton transport mediated with $(n\text{-}Bu_4N)_2$-[4-Fe] is depicted in Scheme 1; a partial dissociation $S_2O_4^{2-} \rightleftarrows 2SO_2^{\cdot-}$ in the W_1 phase generates the active reductant $SO_2^{\cdot-}$ [37] which may reduce $[4\text{-Fe}]^{2-}$ at the W_1/CH_2Cl_2 interface to afford $[4\text{-Fe}]^{3-}$ and SO_2. The former undergoes a protonation probably at sulfur of the Fe_4S_4 core[31] in the CH_2Cl_2 phase when pH_1 is lower than 7.0, and the latter reacts with OH^- to afford HSO_3^- in the W_1 phase. The protonated $[4\text{-Fe}]^{3-}$-H^+ thus formed in the CH_2Cl_2 phase is oxidized by AQS^{2-} at the CH_2Cl_2/W_2 interface with liberating the proton into the W_2 phase. The resulting oxidized cluster $[4\text{-Fe}]^{2-}$ moves back to the W_1/CH_2Cl_2 interface, regenerating $[4\text{-Fe}]^{3-}$-H^+. When pH_1 is higher than 7.0, however, the reduction of $[4\text{-Fe}]^{2-}$ hardly takes place at the W_1/CH_2Cl_2 interface unless a strong hydrophilic Na^+ in the W_1 phase migrates into the CH_2Cl_2 phase to cancel the extra negative charge of $[4\text{-Fe}]^{3-}$. Thus, protons are much more superior to Na^+ as a counter ion of the electron transport in the liquid membrane system. The mechanism proposed in Scheme 1[38] reasonably explains the vectrial electron flow from a high proton concentration side to a low proton concentration side in biomembrane, since the reverse electron transport is effectively suppressed so long as the pK_a value of the cluster is smaller than the pH value of the low proton concentration side. The present study strongly suggests that some of iron-sulfur proteins participate in the transport of protons as well as electrons in biomembrane.

The authors are grateful to the Ministry of Education for support of this work through Grant-in-Aid for Scientific Research (Grant No. 61226007).

References

1) H. T. Tien, *Nature (London)*, **219**, 272 (1968).
2) G. Hauska, *FEBS Lett.*, **79**, 345 (1977).
3) W. E. Ford, J. W. Otvos, and M. Calvin, *Nature (London)*, **274**, 507 (1978).
4) M. S. Tunuli and J. H. Fendler, *J. Am. Chem. Soc.*, **103**, 2507 (1981).
5) I. Tabushi, T. Nishiya, M. Shimomura, T. Kunitake, H. Inokuchi, and T. Yagi, *J. Am. Chem. Soc.*, **106**, 219 (1984).
6) S. S. Anderson, I. G. Lyle, and R. Paterson, *Nature (London)*, **259**, 147 (1976).
7) T. Shinbo, K. Kurihara, Y. Kobatake, and N. Kano, *Nature (London)*, **270**, 277 (1977).
8) J. J. Grimaldi, and J. M. Lehn, *J. Am. Chem. Soc.*, **101**, 1333 (1979).
9) T. Sugimoto, J. Miyazaki, T. Kokubo, S. Tanimoto, M. Okano, and M. Matsumoto, *J. Chem. Soc., Chem. Commun.*, **1981**, 210.
10) J. Devault, *J. Theor. Soc.*, **62**, 115 (1976).
11) P. Mitchell, "Mitochondria/Biomembranes," ed by S. G. Bergh, North Holand, Amsterdam (1972), Vol. 28, p. 358.
12) P. Gerland, R. A. Clegg, J. A. Downie, T. A. Gray, H. G. Lawford, and J. Skyrme, "Mitochondria/Biomembranes," ed by S. G. Bergh, North Holand, Amsterdam (1972), Vol. 28, p. 105.
13) E, Racker and W, Stockenius, *J. Biol. Chem.*, **249**, 662 (1974). N. Sone, Y. Takeuchi, M. Yoshida, and K. Ono, *J. Biochem. (Tokyo)*, **82**, 1751 (1977).
14) P. C. Malony and F. C. Hansen, J. Membr. Biol., **66**, 63 (1982).
15) D. F. Wilson, J. S. Erecinska, and M. Koppelman, *Arch. Biochem. Biophys.*, **151**, 112 (1972).
16) D. F. Wilson, J. G. Lindsay, and E. S. Brockehust, *Biochim. Biophys. Acta*, **256**, 277 (1972).
17) R. F. Urban and M. Klingenberg, *Eur. J. Chem.*, **9**, 519 (1969).
18) "Iron-Sulfur Proteins," ed by T. G. Spiro, Wiley Interscience, New York (1982), Vol. IV.
19) N. A. Stombaugh, J. E. Sundquist, R. H. Burris, and W. H. Orme-Johnson, *Biochemistry*, **15**, 2633 (1976).
20) E. T. Lode, C. L. Murray, and J. C. Rabinowitz, *J. Biol. Chem.*, **251**, 1683 (1976).
21) J. A. Fee, S. G. Mayhew, and G. Palmer, *Biochim. Biophys. Acta*, **245**, 196 (1971).
22) W. J. Ingledew and T. Ohnishi, *Biochem. J.*, **186**, 111 (1980).
23) T. Ohnishi, *Eur. J. Biochem.*, **64**, 91 (1976).
24) R. C. Job and T. C. Bruice, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 2478 (1975).
25) T. C. Bruice, R. Maskiewicz, and R. C. Job, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 231 (1975).
26) B. Odell and P. J. Geary, *J. Chem. Soc., Dalton Trans.*, **1984**, 29.
27) K. Tanaka, T. Tanaka, and I. Kawafune, *Inorg. Chem.*, **23**, 516 (1984).
28) K. Tanaka, M. Moriya, and T. Tanaka, *Inorg. Chem.*, **25**, 835 (1986).
29) K. Tanaka, M. Masanaga, and T. Tanaka, *J. Am. Chem. Soc.*, **108**, 5448 (1986).
30) M. Masanaga, K. Tanaka, and T. Tanaka, *Chem. Lett.*, **1986**, 1531.
31) M. Nakamoto, K. Tanaka, and T. Tanaka, *J. Chem. Soc., Chem. Commun.*, **1986**, 1669.
32) H. Tsai, W. V. Sweeney, and C. L. Coley, *Inorg. Chem.*, **24**, 2796 (1985).
33) Hydroquinone (QH_2) dissociates into QH^-, Q^{2-}, and H^+ in aqueous neutral and alkaline solutions, where Q^{2-} and QH^- may be oxidized by quinone (Q), if it exists, to produce QH · and $Q^{\cdot-}$. Thus, those six species exist as an equilibrium mixture in solutions.
34) The turning point of pH 7.0 in the plot of pH vs. anodic peak potentials has not been changed in the range of sweep rates 10 to 200 mV s^{-1}.
35) The electron transport was conducted at pH_1 6.20 ($NaOH-H_3PO_4$) and pH_2 10.7 ($KOH-H_3PO_4$), and at pH_1 10.7 ($NaOH-H_3PO_4$) and pH_2 10.7 ($KOH-H_3PO_4$).
36) The concentrations of $Na_2S_2O_4$ in the W_1 phase (pH 6.5), $(n\text{-}Bu_4N)_2$[4-Fe] in the CH_2Cl_2 phase, and Na_2AQS in the W_2 phase (pH 10.5) were 1.2×10^{-1}, 7.2×10^{-5}, and 1.0×10^{-1} mol dm^{-3}, respectively.
37) S. G. Mayhew, *Eur. J. Biochem.*, **85**, 535 (1978).
38) The determination of the ratio of e^-/H^+ transported in the present study has been unsuccessful, since a part of SO_2 formed at the W_1/CH_2Cl_2 interface migrates into the W_2 phase through the CH_2Cl_2 phase and reacts with OH^- to afford of a weak acid HSO_3^- (pK_a=7.18) in the W_2 phase, resulting in a difficulty in determination of the amount of H^+ transported owing to significant pH_2 change when the solutions are not buffered.