Dynamic Behaviors of Redox Mediators within the Hydrophobic Layers as an Important Factor for Effective Microbial Fuel Cell Operation

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In a mediator-aided microbial fuel cell, the choice of a proper mediator is one of the most important factors for the development of a better fuel cell system as it transfers electrons from bacteria to the electrode. The electrochemical behaviors within the lipid layer of two representative mediators, thionin and safranine O both of which exhibit reversible electron transfer reactions, were compared with the fuel cell efficiency. Thionin was found to be much more effective than safranine O though it has lower negative formal potential. Cyclic voltammetric and fluorescence spectroscopic analyses indicated that both mediators easily penetrated the lipid layer to pick up the electrons produced inside bacteria. While thionin could pass through the lipid layer, the gradual accumulation of safranine O was observed within the layer. This restricted dynamic behavior of safranine O led to the poor fuel cell operation despite its good negative formal potential.

Key Words : Microbial fuel cell, Redox mediator, Cyclic voltammetry, Phospholipid, Fluorescence

Introduction

Many kinds of chemical fuel cells have been developed to resolve future energy problems.[1] Microbial fuel cells have also been devised as a response to these problems, in which the anodic reaction was replaced by living microorganisms.[2-5] Electrons generated from the catabolic action of microorganisms can be transferred through the cell membrane to the anode. Since the microbial cell membrane generally functions as a barrier to the electron transfer, a proper mediator is needed to increase the fuel cell efficiency.[6,7] Although various microorganism-mediator combinations have been proposed,[8-10] their molecular mechanisms have not been clearly understood. It was believed that the role of the mediator was just believed to transfer electrons produced from the oxidation of substrates to the anode, with the mediator itself undergoing a redox reaction.[10] This paper reports results of electrochemical and fluorescence spectrometric analyses on the dynamic behavior of mediators within the lipid layers. Safranine O and thionin were compared based on their penetration property in the phosphatidylcholine (PC) layers, and this procedure has been used as a model system for the study of a mediator-membrane interaction. Safranine O is a well-known phenazine redox dye and its redox potential is negative enough to produce an appreciable cell potential (Table 1). However, its efficiency as an electron transfer mediator in microbial fuel cells was known to be very feeble.[7] The results obtained in this paper explained why safranine O functioned poorly within a microbial fuel cell in spite of their high negative redox potential.

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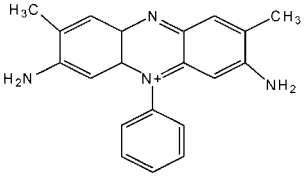
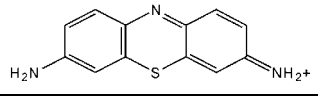
Experimental Section

Chemicals. L-α-phosphatidylcholine (PC), safranine O and 1,6-diphenyl-1,3,5-hexatriene (DPH) were purchased from Sigma (St. Louis, U.S.A.) and thionin from Janssen Chimica (Geel, Belgium). All reagents were used without further purification. Other reagents were of the best quality available.

Preparation of Microorganisms. *Proteus vulgaris* (ATCC 6059) and *Escherichia coli* (ATCC 25922) were obtained from the culture collection of KCCM (Korean Culture Center of Microorganisms) and kept on a nutrient agar plate at 4 °C. Experimental cultures were grown aerobically at 37 °C in a nutrient broth containing 3 g of beef extract and 5 g of peptone per liter. Each cell was harvested by centrifuging at 3,000 × g for 5 min and washed twice with 50 mM phosphate buffer solution (pH 7.0). The washed microorganisms were resuspended in the phosphate buffer to give 20 mg (dry wt) mL^{-1} for the experiment.

Fuel Cell Assembly and Current Output Measurements.

Table 1. Redox mediators, their structural formulae and formal potentials ($E°'$)

Redox mediator	Structural formula	$E°'$ (V)
Safranine O		−0.469
Thionin		−0.117

*Data taken from Figure 2. $E°' = (E_{pa} + E_{pc})/2$.

Each cell unit composed of anode and cathode compartments (internal dimensions 45 × 45 × 15 mm) and separated by a cation exchange membrane (Nafion, Aldrich). A reticulated vitreous carbon (RVC, 30 × 30 × 12 mm) plate was used as an anode. Microorganism and mediators were added to the anodic compartment, and a 50 mM phosphate solution (pH 7.0) was used as an anolyte. A platinum plate (30 × 30 × 0.5 mm) was used as cathode material, and 0.1 M ferricyanide solution as a catholyte.[11] During the experiments, nitrogen was made to flow through the cell compartments for oxygen removal. Operation temperature was maintained at a constant value of 37 °C in a water bath. The cell discharge was performed by the 560-Ω external resistor between an anode and a cathode. The discharge curve was recorded only after the open circuit voltage was stabilized with nitrogen gas flowing through the cell. The cell voltage over time was then recorded with a personal computer equipped with an analogue-to-digital board (Computer Boards, Mansfield, MA, USA). An output current was simply calculated using the ohmic law, $I = V_{cell}/R_{load}$. When the cell voltage dropped to the background level, the cell was charged with a carbon source (1 μmol of glucose) for another discharge measurement. Generally, the cell voltage increased rapidly upon injection of the glucose and reached a plateau level as long as there were enough carbon sources to be consumed by microorganisms, and then the cell voltage began to gradually decrease. The electricity produced was calculated by integrating the discharge curve with time, $Q = \int Idt$.

Electrochemical Analysis. PC was diluted to 2.1 mg/mL with chloroform for the cyclic voltammetric measurements. 0.5 mM solutions of mediators were prepared from 0.1 M phosphate buffer (pH 7.0) using 18 MΩcm^{-1} deionized water. The cast layer of PC was formed by applying 3.0 μL of PC solution onto the glassy carbon (GC) electrode and allowing it to dry. Then the prepared electrode was immediately transferred into each deaerated mediator solution. The conventional three-electrode system (AUTOLAB PGSTAT30) was used to record voltammograms.[12] A platinum wire and an Ag|AgCl|KCl$_{(sat)}$ electrode were used as the counter and the reference electrode, respectively. GC was subjected to polishing with 0.05 μm alumina until there was a mirror finish, and subjected to ultrasonication for 30 sec every time new sets of data were acquired. All the experiments were done under room temperature and atmospheric pressure.

Sample Preparation for Fluorescence Measurements. DPH incorporated unilamellar vesicles formed by extrusion.[19] 10 mg of PC and DPH (molar ratio 500 : 1) were dissolved in chloroform to facilitate mixing, and uniform probe distribution. The chloroform was then evaporated to form a thick film. 1% of α-tocopherol was added to prevent lipid oxidation. Then, the lipid film was dispersed with 50 mM tris-HCl buffer (pH 7.3) and extruded through the 100 nm membrane filter at 50 °C. Particle size of the vesicles was measured by a ZetaPALS particle size analyzer (PSA) in order to confirm the precise vesicle formation. The resulting vesicles were unilamellar, with an average diameter of approximately 140 nm.

Fluorescence Quenching Measurements. Fluorescence emission spectra were measured in a 1 cm path length quartz cuvette using a Hitachi F-2000 spectrofluorimeter at room temperature. The excitation and emission wavelengths were set at 355 nm and 432 nm, respectively. For the quenching process, safranine O and thionin were used as fluorescence quenchers. After the addition of a quencher into DPH-incorporated vesicles, a decrease in the fluorescence signal was recorded. F_o/F, the ratio of the DPH fluorescence intensity in the absence (F_o) to that in the presence (F) of the quencher, was determined.

Results and Discussion

Figure 1 shows typical current-time responses in the microbial fuel cell after the addition of glucose as a carbon source. *P. vulgaris* and *E. coli* were used as model bacteria in this fuel cell system. Thionin exhibited a normal discharge behavior in the fuel cell containing both bacteria, while safranine O gave a very low current output in the same

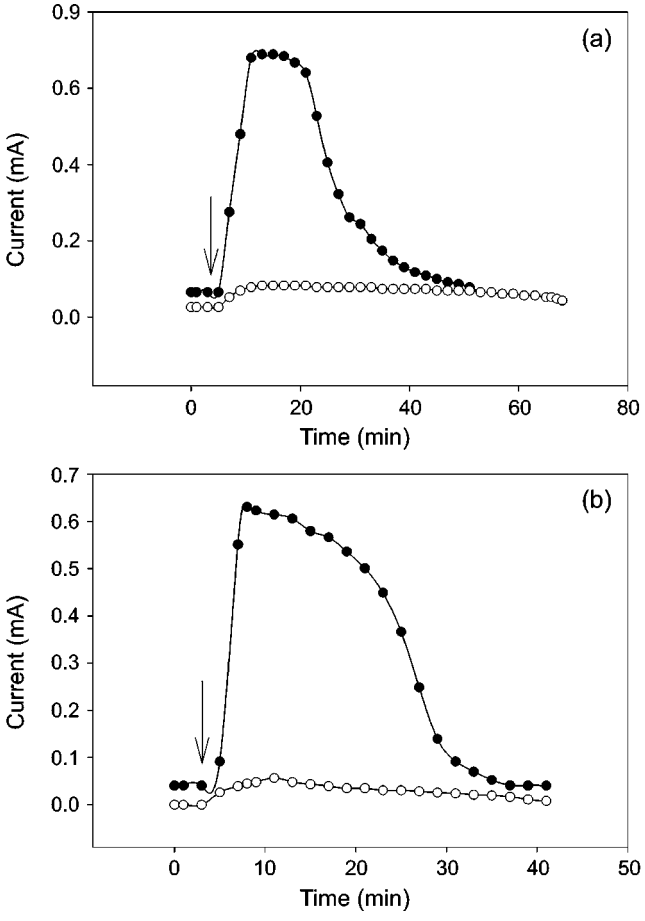


Figure 1. Variation of current output with time through the 560-Ω external load for fuel cells containing (a) *P. vulgaris* and (b) *E. coli* with safranine O (○) and thionin (●) as the electron transfer mediator. Organism concentration: 1 mg (dry wt) mL^{-1}; 1 μmol of mediator and 1 μmol of glucose were added. Addition of glucose is marked by the arrows.

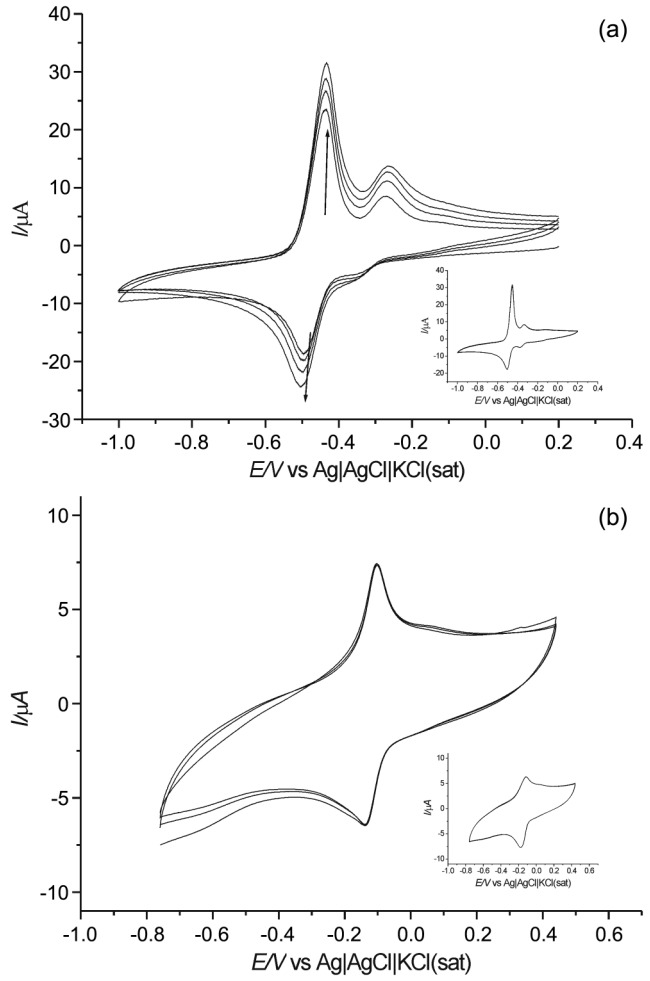


Figure 2. Cyclic voltammograms of safranine O (a) and thionin (b) in the PC film at pH 7.0 solution. Scan rate: 100 $mV \cdot s^{-1}$; Electrode area: 0.071 cm^2. Inset shows the voltammogram on the bare electrode.

operational conditions. These results show that the choice of a proper mediator is a decisive factor in better fuel cell operation. Figure 2 shows cyclic voltammograms of 0.5 mM mediators inside a PC film. 3.0 μL of PC/chloroform solution was applied onto the GC surface and the solvent allowed to evaporate. Compared with bare electrodes (inset), the overall voltammetric shapes remained the same even in the presence of a PC layer which showed a well-defined redox peak. In the case of safranine O, the gradual increase of current, which eventually reached a steady-state value after many cycling, was observed upon the repeated potential cycling, indicating the accumulation of safranine O in the PC layer. But thionin is not readily accumulated in the PC layer since there is a slight increase in current. For an efficient fuel cell operation, both the oxidized and reduced forms of a mediator should freely penetrate the bacterial cell membrane. In the case of safranine O, the oxidized form can go inside the membrane to take up the electrons produced from the oxidation of the substrate, but once reduced inside the cell membrane it resides there, not being able to deliver the electrons to the anode, making the fuel cell efficiency

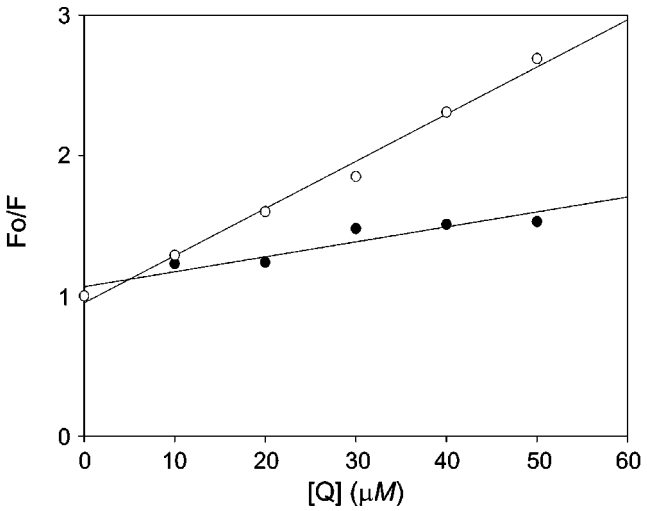


Figure 3. Plot of F_o/F *vs* [Q] according to Stern-Volmer equation in aqueous medium with safranine O (○) and thionin (●). Calculated Stern-Volmer quenching constants (K_{sv}) of mediators are 0.0336 M^{-1} for safranine O and 0.0107 M^{-1} for thionin.

low.[13] The reduced form of thionin, however, can carry the electrons to the anode, passing through the cell membrane.

The penetration property of mediators was also confirmed by the fluorescence quenching study. Fluorescence is a very sensitive technique for understanding the dynamics of fluorescent molecules within a lipid bilayer which composed of amphiphilic molecules such as phospholipids. Information for the location of fluorescence molecules within lipid vesicles can be acquired using quencher molecules because quenching is effective when their distances are closely approached. A DPH molecule was known to be located deep within the lipid bilayers and their fluorescence is enhanced in nonpolar environments such as lipid membranes.[14,15] Since fluorescence emissions are sensitive to circumstances, they are easily quenched by various fluorescence quenchers such as iodide or molecular oxygen.[16] The degree of quenching is inversely proportional to the sixth power of molecular distance,[17] and therefore we can easily examine the dynamic interaction or location of molecules within a lipid layer. The fluorescence quenching of the DPH emission in vesicles with safranine O and thionin was shown in Figure 3. DPH was used as a fluorescence probe, and mediators as quenchers. The Stern-Volmer quenching constant K_{SV} was calculated by plotting F_o/F against Q from the equation[14,18]:

$$F_o/F = K_{SV}[Q] + 1$$

where F_o is DPH fluorescence intensities in the absence of a quencher, F is the intensities in the presence of the quencher, and Q is the concentration of the quencher. A larger K_{SV} means strong quenching of DPH. Since the slope of safranine O is higher than thionin, it can be concluded that DPH was heavily quenched by safranine O. Because the quenching process takes place only at a close range, most safranine O molecules should be found in lipid vesicles or found surviving longer in the DPH located in the lipid layers.

However, thionin did not seem to be fixed within the lipid layers according to the cyclic voltammetric results. The safranine molecule has more lipophilic aromatic moieties, and known to be accumulated by a stacking mechanism on the inner surface of the lipid membrane.[19] The effective mediator in a microbial fuel cell system should be freely and reversibly passed to the lipid layers, yet safranine O accumulated in lipid layers and could not easily escape from there. That is why safranine O was rarely utilized as an electron transfer mediator within a microbial fuel cell in spite of its very negative redox potential. These facts could be explained by their accumulative property in the lipid membranes.

In conclusion, dynamic behaviors as well as electrochemical properties were important factors necessary for an efficient mediator in a microbial fuel cell system. Cyclic voltammetric and fluorescence spectroscopic analyses indicated that both mediators could penetrate lipid layers for the electron transfer, but the gradual accumulation of safranine O was observed within the lipid layers whereas thionin was effectively passing through. This restricted dynamic behavior of safranine O in lipid layers suggested it would be inefficient in a microbial fuel cell system despite its good negative redox potential. These findings will be applied in the further development redox mediators for an efficient microbial fuel cell system.

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References

1. Kordesch, K.; Simander, G. *Fuel Cells and Their Applications*; VCH: New York, 1996.
2. Akiba, T.; Bennetto, H. P.; Stirling, J. L.; Tanaka, K *Biotechnol. Lett.* **1987**, *9*, 611.
3. Allen, R. M.; Bennetto, H. P. *Appl. Biochem. Biotechnol.* **1993**, *39/40*, 27.
4. Choi, Y.; Song, S.; Jung, S.; Kim, S. *J. Microbiol. Biotechnol.* **2001**, *11*, 863.
5. Kim, N.; Choi, Y.; Jung, S.; Kim, S. *Biotechnol. Bioeng.* **2000**, *70*, 109.
6. Kim, H. J.; Hyun, M. S.; Chang, I. S.; Kim, B. H. *J. Microbiol. Biotechnol.* **1999**, *9*, 365.
7. Roller, S. D.; Bennetto, H. P.; Delaney, G. M.; Mason, J. R.; Stirling, J. L.; Thurston, C. F. *J. Chem. Tech. Biotechnol.* **1984**, *34B*, 3.
8. Bennetto, H. P.; Dew, M. E.; Stirling, J. L.; Tanaka, K. *Chem. Indust.* **1981**, *7*, 776.
9. Bennetto, H. P.; Stirling, J. L. *Chem. Indust.* **1985**, *21*, 695.
10. Delaney, G. M.; Bennetto, H. P.; Mason, J. R.; Roller, S. D.; Stirling, J. L.; Thurston, C. F. *J. Chem. Tech. Biotechnol.* **1984**, *34B*, 13.
11. Kaiser, R. D.; London, E. *Biochemistry* **1998**, *37*, 8180.
12. Langner, M.; Hui, S. W. *Chem. Phys. Lipids* **1991**, *60*, 127.
13. Wu, P.; Brand, L. *Anal. Biochem.* **1994**, *218*, 1.
14. Deumie, M.; Baraka, M. E.; Quinones, E. *J. Photochem. Photobiol. A* **1995**, *87*, 105.
15. Kim, N.; Choi, Y.; Jung, S.; Kim, S. *Bull. Korean Chem. Soc.* **2000**, *21*, 44.
16. Won, M. S.; Shim, Y. B.; Park, S. M. *Bull. Korean Chem. Soc.* **1992**, *13*, 680.
17. Kim, S.; Jung, S. *Bull. Korean Chem. Soc.* **1997**, *18*, 1318.
18. Blatt, E.; Sawter, W. H. *Biochim. Biophys. Acta* **1985**, *822*, 43.
19. Bally, M. B.; Hope, M. J.; Van Echteld, C. J. A.; Cullis, P. R. *Biochim. Biophys. Acta* **1985**, *812*, 66.