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Notes

Surface Diluent Effect on the Heterogeneous Electron-transfer Kinetics of PQQ Self-assembled Monolayers

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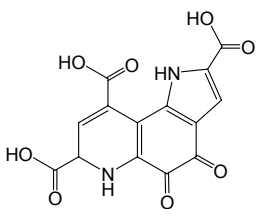
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Self-assembled monolayers (SAMs)[1] have been extensively used as a model system to study long-range electron transfer (ET) kinetics between an electrode and a redox center. They form molecularly flat, well organized surfaces with minimal defects. This feature makes a SAM surface an ideal platform on which fundamental issues on the heterogeneous ET kinetics could be addressed. Other advantages are that complications arising from double layer effect or diffusion process can be avoided. Many systems have been studied. They include ruthenium or osmium complexes,[2,3] ferrocene,[4,5] viologen,[6] azobenzene,[7,8] and various quinone systems.[9-14] Quinones are important as they play an important role in biological systems.[15] Focus has mostly been made on the evaluation of the tunneling barrier constant (β) and the dependency of ET rate constant on the structure of bridges connecting an electrode and an electroactive center. Although it seems that there is a general agreement on the β value of *ca*. 1.0 $Å^{-1}$ per CH_2 unit, different β values have been reported for different quinines.[9,16] The heterogeneous ET rate constant is greatly affected by the nature of bridging molecules. Conjugated bridges give higher ET rate constant compared to unconjugated systems.[17] Also a double-bond bridge gives the fastest ET compared to single- or triple-bond bridges.[18]

In this work, we have studied effects of electroinactive diluents that were coadsorbed with an electroactive SAM on the ET kinetics. Pyrroloquinoline quinone (PQQ) was chosen as an electroactive group. PQQ is found in many dehydrogenases acting as a redox cofactor.[19,20] PQQ has a unique heterocyclic *o*-quinone structure having three carboxylic groups (Scheme 1).

Previously we have shown that the ET kinetics of PQQ was greatly affected by the presence of diluents of fixed chain length.[21] Diluents affected ET rate constants by lessening the lateral interaction between PQQ groups. This work is an extension of the previous study to cover the effect of different alkyl chain lengths of diluents on the ET reactions of coadsorbed PQQ.



Scheme 1. Structure of PQQ

Experimental Section

Materials and reagents. A gold wire (99.999%, 0.5 mm diameter) was obtained from Johnson Matthey (UK). Pyrroloquinoline quinine, alkanethiols (Sigma-Aldrich, USA) and an aminoalkanethiol ($(NH_2(CH_2)_6SH$, Dojindo, Japan) were used as received. All other chemicals were of reagent grade and used without purification.

Monolayer preparation. Working electrodes were made of Au beads (*ca*. 2.1 - 2.4 mm in diameter) formed at the tip of a gold wire (0.5 mm diameter) by melting it in oxygen-gas flame. These bead electrodes were cleaned in a hot "piranha" solution (3:1 mixture of concentrated H_2SO_4 and 30% H_2O_2) for 10 min. After rinsing with copious amount of deionized water, they were electrochemically cleaned by potential cycling in 0.5 M H_2SO_4 in the potential range of −0.20 and 1.50 V *vs*. SCE until a typical voltammogram of clean gold was obtained. This way the surface was maintained very smooth and quite reproducible data could be obtained.

The roughness factor was measured using $Ru(NH_3)_6^{3+}$. The actual surface area obtained from the slope of the linear plot of cathodic peak current *versus* (scan rate)$^{1/2}$ for the reversible reduction was compared with a geometrical area. For the calculation, diffusion coefficient of 7.5×10^{-6} cm^2 s^{-1} at 25 °C was used.[22] The roughness factor was between 1.1 and 1.2.

Fig. 1 shows a series of surface modification steps for a mixed monolayer preparation. A gold bead was thoroughly rinsed with deionized water and ethanol, and dried under nitrogen. Then the electrode was immersed in 1:1 ethanolic mixture of 5 mM of $NH_2(CH_2)_6SH$ and 5 mM of $CH_3(CH_2)_{n-1}SH$ (n = 4, 6, 8, 11) for 2 h. Physically adsorbed compound was removed by rinsing with fresh deionized water. The electrode was treated in solution containing 3 mM of PQQ and 10 mM of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in 10 mM HEPES

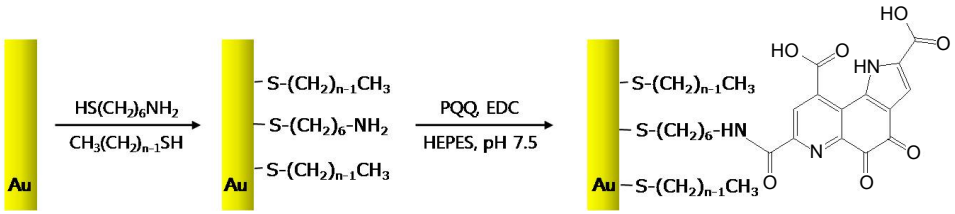


Figure 1. Surface modification steps for the formation of mixed monolayers of PQQ and alkanethiols. n = 4, 6, 8, 11.

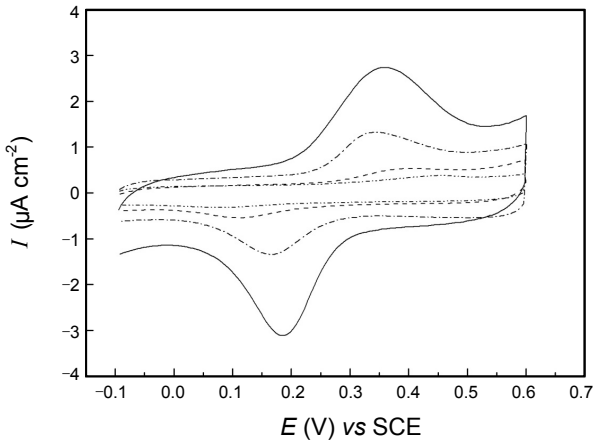


Figure 2. Cyclic voltammograms of PQQ-NH$(CH_2)_6$SH monolayers on gold diluted by butanethiol (solid), hexanethiol (solid dot), octanethiol (dashed), and undecanethiol (solid dot dot) in 0.1 M $HClO_4$. Scan rate = 0.1 V s^{-1}.

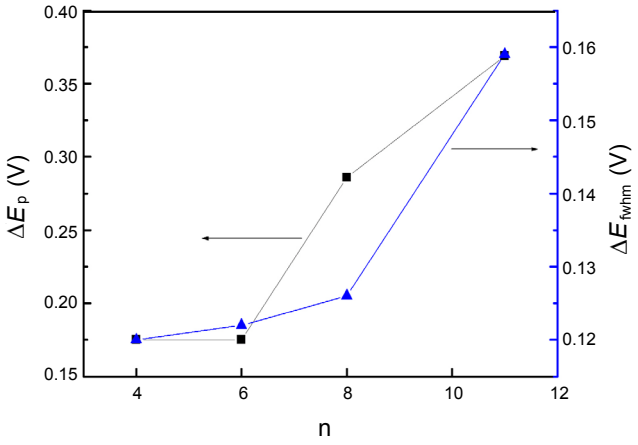


Figure 3. Plot of ΔE_p (■) and ΔE_{fwhm} (▲) *vs* number of carbons (n) in diluent molecules. Data were obtained from Fig. 2.

Table 1. Peak potentials and surface coverage of PQQ-NH$(CH_2)_6$SH diluted by $CH_3(CH_2)_{n-1}SH$ (n = 4, 6, 8, 11) on gold

n	E_{pa} (V)	E_{pc} (V)	ΔE_p (V)	ΔE_{fwhm} (V)	Γ (nmol cm^{-2})	Γ/Γ_{full}
4	0.360	0.185	0.175	0.120	0.14	0.61
6	0.342	0.167	0.175	0.122	0.067	0.29
8	0.397	0.111	0.286	0.126	0.037	0.16
11	0.455	0.086	0.369	0.159	0.019	0.082

buffer (pH = 7.5) for 1 h to attach PQQ on the top of the amine group. Thus prepared PQQ-modified electrode was again rinsed in deionized water to remove the physically adsorbed PQQ before voltammetric study.

Measurements. Electrochemical measurements were carried out in a single compartment cell using a three-electrode system composed of a Au bead, a platinum wire, and SCE as working, counter, and reference electrodes, respectively. Voltammetric experiments were performed with a potentiostat (Autolab PG-STAT 30, ECO CHEM). All the solutions were prepared with deionized water with resistivity of 18 MΩ cm or more purified by AquaMax-Ultra system (Younglin, Korea) and deaerated by purging with nitrogen. All measurements were carried out at room temperature.

Results and Discussion

Electrochemistry of mixed monolayers. Fig. 2 shows typical cyclic voltammograms (CVs) of PQQ-NH$(CH_2)_6$SH diluted by different alkanethiols, $CH_3(CH_2)_{n-1}SH$ (n = 4, 6, 8, 11) on gold. The redox waves found at *ca*. 0.35 and 0.18 V are due to the two-proton, two-electron process, PQQ + $2H^+$ + $2e^-$ → $PQQH_2$.[23] The peak currents in all four cases were linearly proportional to the scan rate indicating that PQQ was securely confined on the surface, and unchanged over the potential cycling. The surface coverage (Γ) of PQQ was calculated from the charges under the voltammetric waves. Although mixed monolayers were prepared from 1:1 ethanolic mixture, the Γ values are less than half of that of a full monolayer (Γ_{full}), except for n = 4 (Table 1). The coverage decrease with the increase in alkyl chain length is ascribed to the fact that alkanethiols of a longer chain length tend to form a more well-ordered and well-packed monolayer than those of a shorter chain length. For n = 11, only 8.5% of the surface is covered with PQQ. The coverage of a full monolayer was obtained without dilution.

Some voltammetric characteristics should be noted. Reversibility depends on the chain length of a diluent. ΔE_p increases with increase in the chain length (Fig. 3). ΔE_p for n = 4 and 6 was 175 mV but it increased to 286 and 369 mV for n = 8 and 11, respectively. Full width at half maximum (ΔE_{fwhm}) can also be an indicator for the reversibility. As the chain length increases from 4 to 11, ΔE_{fwhm} increases from 120 to 159 mV accordingly. The increasing irreversibility with the chain length of diluents is surprising because it is expected that electron transfer kinetics is essentially independent of the presence of diluents under ideal conditions. With lateral interactions between electroactive head groups present, reversibility would be affected.[24] Less

Table 2. Electrochemical kinetic parameters of PQQ-NH$(CH_2)_6$S/Au diluted by $CH_3(CH_2)_{n-1}SH$ (n = 4, 6, 8, 11) in 0.1 M $HClO_4$

n	αn	(1-α)n	log k_{app} (s^{-1})
4	1.22	0.78	−1.98
6	1.28	0.85	−2.26
8	0.95	0.53	−2.57
11	0.83	0.69	−4.10

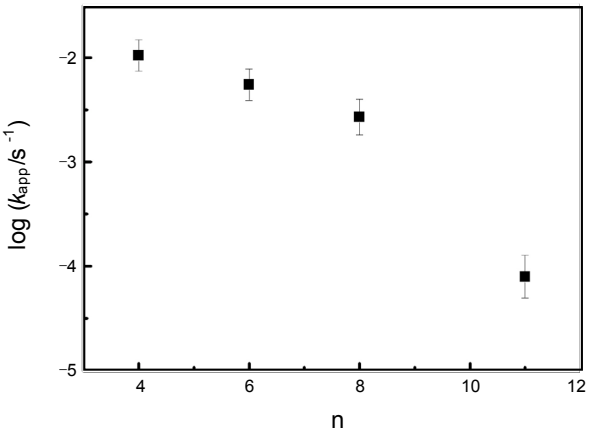


Figure 4. Dependence of apparent rate constant on the number of carbons (n) in diluent molecules.

repulsive lateral interaction upon dilution results in improved reversibility. The present results, however, shows exactly the opposite. More dilution by using a longer alkanethiol caused larger ΔE_p and ΔE_{fwhm}. If the dilution only affects lateral interactions, more dilution would have resulted in better reversibility. This strongly implies that there must be other factors influencing electrochemical reactions of PQQ.

In their mixed monolayer experiments for naphthoquinone SAMs, Bulovas *et al*.[25] have shown that proton accessibility to the quinone group played an important role in the electrochemical response. When a diluent was long enough to cover the quinone group preventing proton access to the surface from the bulk, a broad, ill-defined voltammograms were resulted. However, when a carboxyl group-terminated thiol was used, reversibility was recovered, indicating that protons from dissociation of carboxyl groups participated in the reaction. The same argument may hold for our case. The presence of a diluent makes local pH around PQQ moiety different from that of the bulk, hindering protons from having access to the surface. This hindering effect will be greater for the diluent having a longer alkyl chain length. For n = 4 or 6, the PQQ moiety is well above the background diluents so that the voltammetric features are not much affected. For n = 8 and 11, however, proton accessibility is much hindered, causing a local pH around PQQ to be higher than that of the bulk. This hindering effect becomes more and more pronounced and eventually affects electron transfer kinetics as the chain length increases.

Electron transfer kinetics. Finite electron transfer rate causes peak potential shift with scan rate. According to Laviron,[26] standard rate constant and the electron transfer coefficient could be extracted from the cyclic voltammograms by the following equations.

$$E_{pc} = E_c^{o'} - (RT/\alpha nF) \ln[\alpha nF\, \nu_c/RTk_{app}] \quad (1)$$

$$E_{pa} = E_a^{o'} - (RT/(1-\alpha)nF) \ln[(1-\alpha)nF\, \nu_a/RTk_{app}] \quad (2)$$

where α is the transfer coefficient, ν the sweep rate, k_{app} the apparent rate constant, and R, T, and F have their usual meanings. ν_c and ν_a are the critical scan rates obtained by extrapolating the linear portion of the E_p vs ln(ν) plots to the formal cathodic and anodic potentials $E_c^{o'}$ and $E_a^{o'}$. The slopes of the linear portion of the E_p *vs*. ln(ν) curves are $RT/\alpha nF$ and $RT/(1-\alpha)nF$ for the cathodic and anodic branches, respectively. αn and $(1-\alpha)n$ values obtained from each slope were substituted to eqs 1 and 2 to solve for k_{app}. The two apparent rate constants obtained in this way were averaged. Table 2 lists αn and $(1-\alpha)n$ values along with k_{app} for different chain lengths. αn and $(1-\alpha)n$ values are not equal, indicating the energy barrier for the redox reactions of PQQ is not symmetrical regardless of diluents.

Fig. 4 shows the plot of the logarithmic heterogeneous apparent rate constant as a function of the number of carbons (n) in diluent molecules. With the increase in the chain length, the electron transfer rate becomes slower, which is the cause of the voltammetric irreversibility (see above). A slight decrease in k_{app} up to $n = 8$ underwent abrupt drop for $n = 11$. This can be explained in terms of proton availability. The total length of PQQ-NH$(CH_2)_6$SH is 20.6 Å. The *o*-quinone part of PQQ is about 13 Å above the surface so that butanethiol (6.5 Å) and hexanethiols (9.8 Å) cannot effectively hinder the proton access to the PQQ moiety. A slight decrease in k_{app} may indicate that PQQ-NH$(CH_2)_6$SH is little bent. When an octanethiol (12.3 Å) was used, proton availability is believed to be somewhat limited, making k_{app} further decrease.

It has been well documented that proton concentration affects apparent rate constant when electron transfer is coupled with protons. Laviron[27-30] formulated a nine-member square scheme for two-electron, two-proton reactions for quinone/hydroquinone redox reactions, assuming that protonation is at equilibrium. Later Finklea[31] extended Laviron's work to include electron transfer coefficients varying with applied potential. The theory predicts a 'W'-shape curve when the rate constant is plotted against pH with two minima near neutral pHs. Thus higher local proton concentration should give rise to higher rate constant in an acidic branch. The exact evaluation of local pH, however, is possible only when all six pK_a values and six formal potentials for quinone/hydroquinone are known.[32] Lower rate constants for longer diluents clearly indicate lower proton concentration around the PQQ moiety due to hydrophobic environment of alkyl chains. Although it is not possible to obtain exact local proton concentration, it may be at least two orders of magnitude lower for undecanethiol than for butanethiol, estimated from the apparent rate constants.

In this paper, we have formed a PQQ monolayer diluted by alkanethiols of different chain length and showed electrochemical reversibility and electron transfer rate constant are greatly affected by the local proton concentration near the electroactive

group when protons are coupled with electrochemical reactions. By blocking the proton access to the PQQ moiety, the redox reaction of PQQ becomes irreversible and electron transfer rate slower accordingly.

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References

1. Ulman, A. *An Introduction to Ultrathin Organic Films: From Langmuir-Blodgett to Self-Assembly*; Academic Press: San Diego, 1991.
2. Weaver, M. J.; Li, T. *J. Am. Chem. Soc.* **1984**, *106*, 6107.
3. Foster, R. J.; Faulkner, L. R. *J. Am. Chem. Soc.* **1994**, *116*, 5444.
4. Chidsey, C. D. E. *Science* 1**991**, *51*, 919.
5. Smalley, J. F.; Feldberg, S. W.; Chidsey, C. D. E.; Linford, M. R.; Newton, M. D.; Liu, Y.-P. *J. Phys. Chem.* **1995**, *99*, 1314.
6. Finklea,H. O.; Hanshew, D. D. *J. Am. Chem. Soc.* **1992**, *114*, 3173.
7. Yu, H. Z.; Shao, H. B.; Luo, Y.; Zhang, H. L.; Liu, Z. F. *Langmuir* **1997**, *13*, 5774.
8. Wang, Y. Q.; Yu, H. Z.; Cheng, J. Z.; Zhao, J. W.; Cai, S. M.; Liu, Z. F. *Langmuir* **1996**, *12*, 5466.
9. Mukae, F.; Takemura, H.; Takehara, K. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 246l.
10. Zhang, L.; Lu, T.; Gokel, G. W.; Kaifer, A. E. *Langmuir* **1993**, *9*, 786.
11. Hong, H.-G.; Park, W. *Langmuir* **2001**, *17*, 2485.
12. Hong, H.-G.; Park, W.; Yu, E. *J. Electroanal. Chem.* **1999**, *476*, 177.
13. Hong, H.-G.; Park, W.; Yu, E. *Bull. Korean Chem. Soc.* **2000**, *21*, 23.
14. Tramell, S. A.; Lowry, D. A.; Seferos, D. S.; Moore, M.; Bazan, G. D.; Lebedev, N. *J. Electroanal. Chem.* **2007**, *606*, 33.
15. Chambers, J. Q. *The Chemistry of the Quinonoid Compounds*; Patai, S., Ed.; Wiley: New York, 1974; 737-792.
16. Nagata, M.; Konda, M.; Suemori, Y.; Ochiai, T.; Dewa, T.; Ohtsuka, T.; Nango, M. *Colloids Surf. B: Biointerfaces* **2008**, *64*, 16.
17. Trammell, S. A.; Seferos, D. S.; Moore, M.; Lowy, D. A.; Bazan, G. C.; Kushmerick, J. G.; Lebedev, N. *Langmuir* **2007**, *23*, 942.
18. Trammell, S. A.; Moore, M.; Schull, T. L.; Lebedev, N. *J. Electroanal. Chem.* **2009**, *628*, 125.
19. Duine, J. A. *Eur. J. Biochem.* **1991**, *200*, 271.
20. Jongejan, J. A., Duine, J. A., Eds.; *PQQ and Quinoproteins*; Kluwer: Dordrecht, 1988.
21. Park, W.; Ahmed, J.; Kim, S. *Colloids Surf. B: Biointerfaces* **2009**, *68*, 120.
22. Gomez, M. E.; Li, J.; Kaifer, A. E. *Langmuir* **1991**, *7*, 1797.
23 Zayats, M.; Katz, E.; Baron, R.; Willner, I. *J. Am. Chem. Soc.* **2005**, *127*, 12400.
24. Matsuda, H.; Aoki, K.; Tokuda, K. *J. Electroanal. Chem.* **1987**, *217*, 15.
25. Bulovas, A.; Dirvianskyte, N.; Talaikyte, Z.; Niaura, G.; Valentukonyte, S.; Butkus, E.; Razumas, V. *J. Electroanal. Chem.* **2006**, *591*, 175.
26. Laviron, E. *J. Electroanal. Chem.* **1979**, *101*, 19.
27. Laviron, E. *J. Electroanal. Chem.* **1981**, *124*, 1.
28. Laviron, E. *J. Electroanal. Chem.* **1981**, *124*, 9.
29. Laviron, E. *J. Electroanal. Chem.* **1983**, *146*, 1.
30. Laviron, E. *J. Electroanal. Chem.* **1983**, *146*, 15.
31. Finklea, H. O. *J. Phys. Chem. B* **2001**, *105*, 8685.
32. Trammell, S. A.; Lebedev, N. *J. Electroanal. Chem.* **2009**, *632*, 127.