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Electrochemistry of Cytochrome *c* at a Lipid Langmuir Blodgett Monolayer Electrode

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Cytochrome *c* was found to undergo electron transfer reactions at a gold electrode modified with a Langmuir-Blodgett monolayer of 20-mercaptoeicosane-1-ol/dioleoyl-L-α-phosphatidylcholine (molar ratio, 1/20). The stability of the monolayer and the adsorption behavior of cytochrome *c* to the monolayer was also examined.

Since the finding (in 1977)[1-4] that redox proteins could communicate with electrodes, a variety of reports on the electrochemistry of proteins have been published.[5] Cytochrome *c*(cyt *c*), which is a well-known water-soluble heme protein that functions as an electron carrier between cyt *c* reductase and cyt *c* oxidase at the inner bilayer membrane of the mitochondria, is reported to show reversible voltammetric responses at gold electrodes that were modified with so-called promoters such as bis (4-pyridyl) disulfide.[6] We and others have been interested in the electrochemistry of cyt *c* and related redox-active proteins at molecular-membrane electrodes from the standpoint that these are biological membrane-related proteins.[7-11] Understanding of lipid membrane/protein interactions at electrode systems should be important both in fundamentals of biomembrane functions and applications such as the construction of membrane/protein devices. In this communication we would like to report the finding that the electrochemistry of cyt *c* at a lipid Langmuir-Blodgett (LB) monolayer electrode is possible. Here, dioleoyl-L-α-phosphatidylcholine (DOPC) was used as a LB monolayer forming lipid and 5 mol% of 20-mercaptoeicosane-1-ol ($HSC_{20}OH$) was added to DOPC for the stability of the monolayer on the electrode.

20-Mercaptoeicosane-1-ol was synthesized by using SL-20 (a mixture of 1,20-eicosanedioic acid and 1,16-hexadecanedioic acid) as a starting material. Separation of 1,20-eicosanedioic acid was conducted in an alkaline slurry state of SL-20. 1,20-Eicosanediol was obtained by the usual esterification of the dicarboxylic acid followed by the reduction with $LiAlH_4$. Bromination reaction (solvent, ligroin) of the diol with a liquid-liquid extraction apparatus gave 20-bromoeicosane-1-ol (mp 63-65 °C) which was reacted with thiobenzoic acid in ethanol at reflex temperature under a N_2 atmosphere. The crude product was recrystallized from ethanol: yield 50%, mp 73-75 °C. Finally, 20-mercapto-eicosane-1-ol ($HSC_{20}OH$)[12] was obtained by the reduction with hydrazine in ethanol. Horse heart ferricytochrome *c* (type VI, Sigma Chemical Co.,) was purified by chromatography on carboxymethylcellulose (CM-52, Whatman) according to a published procedure.[13] DOPC was purchased from Sigma Chemical Co., and was used without further purification.

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DOPC/$HSC_{20}OH$ (molar ratio, 20/1) was dissolved in benzene/ chloroform(16/1 v/v) and was spread on the air–water (60 mM phosphate buffer) surface of a computer-controlled Langmuir trough (USI System Co. Ltd., model FSD-20 and FSD-23). After evaporating the solvent, the monolayer on the water was compressed to a surface pressure of 30 mN/m. An almmina-polished gold disk electrode (diameter, 1.6 mm) was slowly immersed horizontally through the monolayer into the trough in order to deposit the monolayer onto the Au. The LB monolayer electrode thus obtained was assembled in an electrochemical cell without any contact of the monolayer with air. Cyclic voltammetry (equipment: Bioanalytical Systems, Electrochemical Analyzer, type 100B) was conducted in 60 mM phosphate buffer at 20 °C under nitrogen atmosphere. A saturated calomel electrode (SCE) and a platinum wire were used as the reference and the counter electrode, respectively.

Figure 1 shows a cyclic voltammogram (CV) of the DOPC/$HSC_{20}OH$ LB monolayer electrode in the presence of 2 mmol dm^{-3} $K_3Fe(CN)_6$. In general, it is not easy for LB monolayers on electrodes to block the electrochemistry of such small redox molecule, because many monolayers are not homogeneous in structures but often possessing defects as shown recently by the direct observation of monolayer phases on air-water interfaces with an optical microscopic technique.[14] The LB monolayer of DOPC/ $HSC_{20}OH$, however, fairly blocked the redox reaction of $Fe(CN)_6^{3-}$, indicating that the coverage of the electrode with this monolayer is high. When ferricyanide was replaced with 100 μmol dm^{-3} cyt *c*, well-defined voltammograms with $E^{\circ\prime}$=14–17 mV were observed (Fig. 2). The ion size of cyt *c* is much larger than that of $Fe(CN)_6^{3-}$, therefore it

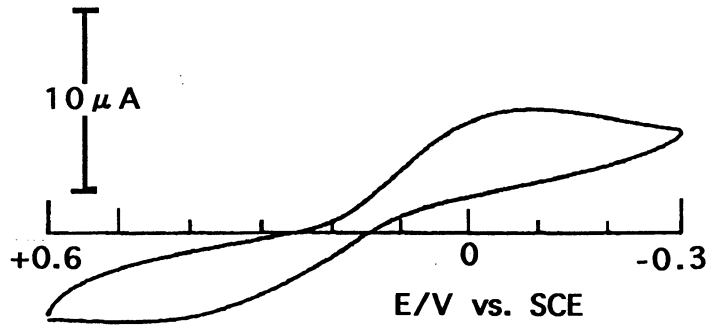

Fig. 1. Cyclic voltammogram of the DOPC/$HSC_{20}OH$ (molar ratio, 20/1) LB monolayer electrode in 60 mmol dm^{-3} phosphate buffer (pH 7.0) at 20 °C. The solution contains 2.0 mmol dm^{-3} $K_3Fe(CN)_6$ and 0.1 mol dm^{-3} KCl. Scan rate, 200 mV s^{-1}.

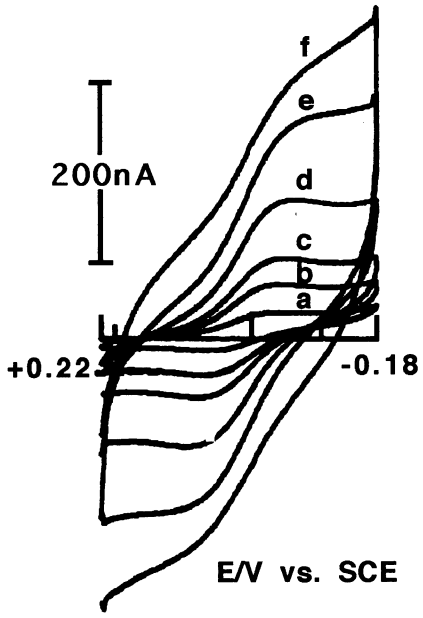

Fig. 2. Cyclic voltammograms of 100 μmol dm^{-3} cyt *c* at the DOPC/$HSC_{20}OH$ LB monolayer in a 60 mmol dm^{-3} phosphate buffer (pH 7.0) at 20 °C. Scan rates are: (a) 2, (b) 10, (c) 20, (d) 50, (e)100 and (f) 200 mV s^{-1}.

can not be assumed that observed CVs of cyt *c* are from the electrochemical communication of cyt *c* with an unmodified area of the electrode. The measured formal potentials are almost consistent with those for elecatrodes modified with electron promoter compounds.[6] Both cathodic and anodic peak currents of the voltammograms are proportional to the square root of the scan rate for 2–100 mV s^{-1}, indicating that the electrochemistry is controlled by the diffusion of cyt *c* in the solution.

The blocking ability of the LB monolayer was examined again by the replacement of cyt *c* with ferricyanide and found that a CV was almost the same as in Fig. 1.

The stability of the monolayer and the adsorption of cyt *c* to the monolayer was examined by a time course experiment after the replacement of ferricyanide with cyt *c*. Cyclic voltammograms of the LB monolayer which was kept for 24 h at 5 °C were shown in Fig. 3. Apparently the current intensity decreased. Blocking ability of the monolayer toward ferricyanide at this stage was found increase compared to the fresh monolayer (Fig. 4). However, when ferricyanide was replaced again to cyt *c*, the intensity of obtained CV was almost the same as in Fig. 1. Similar results were obtained for the monolayer electrode which was stored at 5 °C for one week and three weeks. These results indicate that: i) cyt *c* adsorbs gradually to the surface of the lipid monolayer and the adsorbed cyt *c* prohibit the electron transfer of cyt *c* in the solution, ii) weakly adsorbed cyt *c* is removed by the replacement process to the redoxactive species, iii) the electrochemistry of adsorbed cyt *c* at the monolayer is not observed, iv) the lipid LB monolayer on the electrode is stable in the buffer solution for more than three weeks.

In conclusion, we have found for the first time that the electrochemistry of cyt *c* at the lipid LB monolayer electrode is possible. The schematic illustration for two possible electron transfer

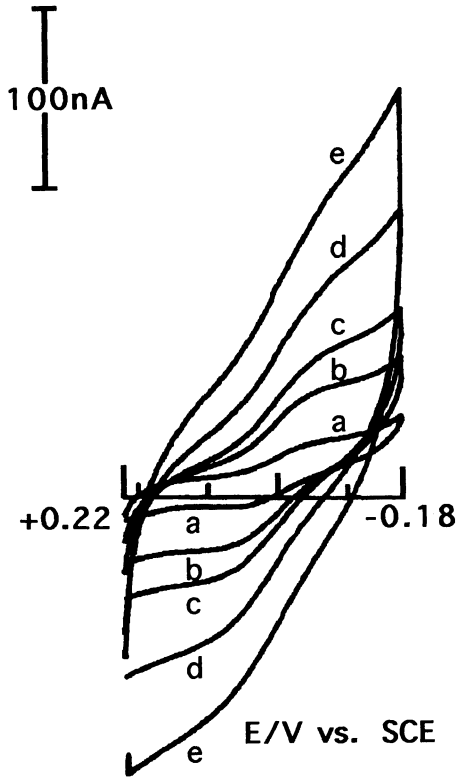

Fig. 3. Cyclic voltammograms for the DOPC/$HSC_{20}OH$ LB monolayer which was stored at 5 °C for 24 h in the presence of cyt *c*. Scan rates (mVs^{-1}) are: (a) 2, (b) 10, (c) 20, (d) 50 and (e) 100. Temperature, 20 °C.

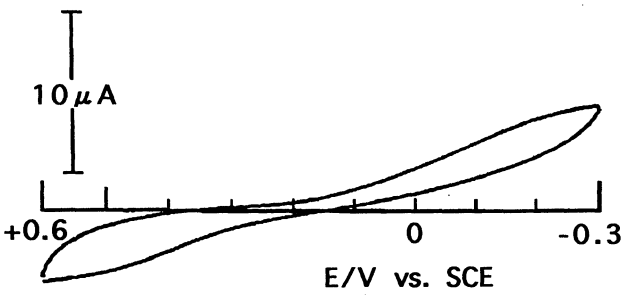

Fig. 4. Cyclic voltammogram of the DOPC/ $HSC_{20}OH$ LB monolayer electrode after the measurement of Fig. 3; here cyt *c* is replaced with 2.0 mmol dm^{-3} $K_3Fe(CN)_6$. Scan rate, 200 mV s^{-1}. 20 °C.

mechanisms is given in Fig. 5; i.e. one is the long distance electron transfer via the lipid monolayer on gold (Fig. 5, above) and the other is the electron transfer of cyt *c* diffused into the inner space of the monolayer (Fig. 5, below). Further electrochemical characterizations of this electrode systems are underway in our laboratory.

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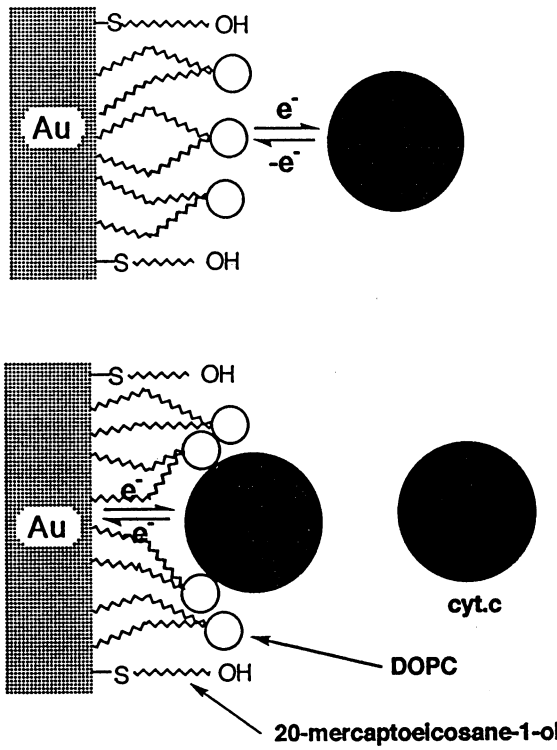

Fig. 5. Possible structure for the lipid monolayer gold electrode / cyt *c* solution interface.

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