Electrochemical Analysis in a Liposome Suspension Using Lapachol as a Hydrophobic Electro Active Species

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This study demonstrated that the electro-chemical analysis of hydrophobic quinones can be performed in liposome suspension systems. We prepared and analyzed liposome suspensions containing lapachol, which is a quinone-based anti-tumor activity compound. In this suspension system, a simple one redox couple of lapachol is observed. These results are quite different from those obtained in organic solvents. In addition, the pH dependence of redox behaviors of lapachol could be observed in multilamellar vesicle (MLV) suspension system. This MLV suspension system method may approximate the electrochemical behavior of hydrophobic compounds in aqueous conditions. A benefit of this liposome suspension system for electrochemical analysis is that it enables to observe water-insoluble compounds without using organic solvents.

Key words cyclic voltammogram; lapachol; liposome; multilamellar vesicle (MLV)

Electrochemical analyses of organic compounds with cyclic voltammetry or pulse voltammetry provide us general information about the redox property of those compounds. Commonly, organic solvents are used for the analysis of organic compounds with an excessive amount of supporting electrolytes such as tetraalkyl ammonium salts. The combination of an organic solvent with an electrolyte enables it to analyze electrochemical behaviors of organic compounds. However, the results highly depend on the stabilization energy from organic solvents and electrolytes, especially redox potentials are very sensitive to electrolyte concentrations.1,2) This mutual dependence sometimes causes problems in understanding the meaning of the potentials.

On the other hand, quinones play an important role in biological systems and serve as active sites for quinonenzymes in biological functions. It is presumed that the redox properties of quinones are controlled by hydrogen bonding with surrounding proteins or solvents. As a result, hydrogen-bonding interactions have been the primary focused of most studies.3,5) Most studies are performed using quinone compounds and proton donors in coexistence with organic solvents, so as to increase the solubility. However, it is unclear whether the results of an electrochemical measurement using organic solvents reflect real in vivo behavior.

We propose that liposome suspensions might serve as a substitute for organic solvents. Liposomes have been studied as an artificial biomembranes and also play important roles as drug delivery systems. The possibility of liposomal use diverges into many scientific branches. Recently, an interesting study reported that electrochemical measurements were performed using a liposome-modified electrode.6,7) On the basis of these studies, we attempted to perform electrochemical measurements using a liposome suspension in place of organic solvents.

Results and Discussion

Preparation of a Liposome Suspension Using a Hydrophobic Electro-Active Compound First, we tried to prepare electro-active liposome suspensions. A chloroform solution containing soy lecithin (200mg) is taken in a round-bottom flask and evaporated under reduced pressure to remove the solvent. Then, a thin film of lecithin is obtained on the surface of the round-bottom flask. Next, 0.1m (pH 7) phosphate buffer (20mL) is poured into the round-bottom flask and mixed using a vortex mixer warming at ca. 50°C. From this, we obtain a white colored multilamellar vesicle (MLV) suspension.5) The MLV suspension is very stable and does not precipitate throughout the day. Figure 1 shows an optical microscope photograph of the MLV suspension, prepared by the method mentioned above. It illustrates spherical MLVs, which have an average diameter of ca. 5µm. In similar manner, liposome suspensions are prepared, which contain a hydrophobic compound in the buffer solution. Lapachol, a water-insoluble quinone, is selected as a hydrophobic compound.9) Lapachol is a quinone-based anti-tumor compound derived from natural products.10–12) Most quinone system anticancer agents show activity by an active oxygen species produced by redox processes. Because of this, the electrochemical behavior of lapachol has been extensively studied since 2002.13–15) Similar to the method mentioned above, chloroform solutions containing both soy lecithin (200mg) and lapachol (0.02mmol)
MLV-lapachol particles is not uniform because of these non-uniform sizes, however, the influence on CV measurement could be ignored.

Next, we made thin films of lecithin (200 mg) with various amount of lapachol, and then prepared several MLV-lapachol suspensions. Figure 3 shows the CVs of MLV-lapachol suspensions that include different amounts of lapachol. With increasing lapachol concentrations, the cathodic peak current increases. A linear relationship between the peak current and the lapachol concentration is apparent. However, the cathodic peak current is smaller than expected from the lapachol concentration. One reason for this is that the diffusion coefficient of MLV-lapachol is much smaller than lapachol itself. Furthermore, it is believed that lapachol exists in the inner layer of the MLV, indicating that the distance to the electrode is too far for redox reactions to occur.

The pH Dependence of CVs of MLV-Lapachol Suspensions vs. CVs of Lapachol in CH$_3$CN with Acetic Acid

Figure 4 shows the changes in CVs of the MLV-lapachol suspension with the addition of different amount of 10% NaOH solutions. The CV, represented as a solid line, corresponds to MLV-lapachol in pH 7 phosphate buffer. When 10% NaOH is added, the pH increases, and the redox wave is shifted in a negative direction. This behavior indicates that protons participate in the redox reaction. Figure 4 shows a relationship between the cathodic peak potential and pH of the suspensions. The dashed line is the linear regression analysis and the slope shows $-57.7$ mV/pH. It suggested that the cathodic peak potential moves negative direction according to the Nernst equation. In the case of around pH 10, it was difficult to do experiment because of the less buffering ability. On the other hand, by increasing the pH value, the color of the MLV-lapachol suspension becomes orange red. This color change indicates the dissociation of the OH group, and the stability of the MLV-lapachol suspension decreased for pH over 11. Regardless, the stability during the measurement did not cause a problem.

Next, we measured CVs of lapachol in organic solvent
mixed with proton donors, and the results were compared with those of MLV-lapachol. Figure 5 shows the changes in CVs of lapachol with increasing concentrations of acetic acid in dry CH$_3$CN. In the absence of acetic acid, lapachol shows complicated redox behaviors (Fig. 5(1)). These are one electron (the first wave) and two electron (the second wave) reactions.\textsuperscript{13–15} Increasing the concentration of acetic acid shifts the second wave in a positive direction and merges it into the first wave. When the concentration of acetic acid reaches 10 mM (Fig. 5(5)), the cathodic current value is three times larger than the first cathodic peak current of Fig. 5(1). Furthermore, when acetic acid is added, the redox couple is shifted positively, and the cathodic current is decreased. In the presence of excess acetic acid (ca. 5 M), the CV shows a 2e$^-$/2H$^+$ reaction wave. When comparing the results of MLV-lapachol (Fig. 2b) with the CV of lapachol in CH$_3$CN containing 5 M acetic acid (Fig. 5(8)), these shape of CV are similar but not same.

The electrochemical behavior of lapachol has been studied in organic solvent because of the solubility. In this work, we prepared MLV-lapachol suspensions and performed electrochemical measurements of those, and then it was shown that lapachol is easily reduced in pH 7 buffer solution. In addition, the pH dependence of redox behaviors of lapachol could be observed in MLV suspension system. This MLV suspension system method may approximate the electrochemical behavior of hydrophobic compounds in aqueous conditions. Also a benefit of this suspension system for electrochemical analysis is that it enables to observe water-insoluble compounds without using organic solvents.

**Experimental**

Lecithin from soybeans, chloroform (99%) and acetic acid (99%) are commercially available (Nacalai Tesque, Inc.) and are used as received without further purification. Lapachol and dehydrated CH$_3$CN of the best available grade from Aldrich Chemical Co. are used. Cyclic voltammograms are recorded at room temperature (ca. 25°C) under nitrogen atmosphere with an ALS 600C model electroanalytical system (BAS, Inc.) using a three electrode system consisting of a GC disk electrode with a diameter of 3 mm as a working electrode, a platinum wire as a counter electrode, and a Ag/Ag$^+$ reference electrode (BAS, Inc.). Further details of our electrochemical measurements are described in a previous paper.\textsuperscript{5}

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**References and Notes**


9) Lapachol is insoluble in water (under pH 7, at room temperature condition), soluble in alcohol or aqueous alkali solution.


16) In liposome suspensions, concentrations of lapachol are estimated as an amount of lapachol divided by total buffer volume. For example, when 0.1 M phosphate buffer (20 mL) is added into a round-bottom flask with thin film of soy lecithin and lapachol (0.020 mmol), the concentrations of lapachol are treated as 1.0 mM.