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Optimal-Fabrication Conditions of Cytochrome *c* LB Films

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The optimal fabrication condition of cytochrome *c* Langmuir-Blodgett (LB) film was investigated. To verify the pH dependence of cytochrome *c* film formation, the various π-A isotherms were obtained at pH 6.0, 7.0, 8.0, 9.0, and 10.0, respectively. The cytochrome *c* LB films were then fabricated onto the quartz substrate, and the deposition of cytochrome *c* LB films was investigated by UV-visible spectroscopy.

Keywords: Cytochrome *c* (Cyt-*c*); Langmuir-Blodgett (LB); UV-visible spectroscopy

INTRODUCTION

Cytochrome *c* is one of the most widely studied proteins due to its stability and solubility in water as well as an electron transport property [1]. Since cytochrome *c* act as an electron transport component in the bacterial photosynthetic reaction center, it is very reasonable approach to use cytochrome *c* as an electron acceptor in the development of molecular electronic device by mimicking biological photosynthesis mechanism. In order to achieve this, the fabrication of cytochrome *c* films is considered as the most important process in the development of bioelectronic device. However, it is difficult to directly fabricate molecular thin film onto substrate generally because cytochrome *c* is water-soluble protein. So, the film formation

conditions are the important factors to fabricate the optimal molecular thin film consisting of cytochrome *c*.

In this study, we investigated the cytochrome *c* Langmuir-Blodgett (LB) film formation at various pHs and determined the optimal condition for the cytochrome *c* LB film fabrication.

EXPERIMENTAL DETAILS

Cytochrome *c* extracted from horse heart (type IV) was purchased from Sigma Chemical Company. The other chemicals used in this study were obtained commercially as the reagent grade. Cytochrome *c* was dissolved in phosphate buffer solution of pH 6.0, 7.0, 8.0, 9.0, and 10.0, respectively. For spreading the cytochrome *c* solution (4.0mM) onto the subphase, cytochrome *c* solutions with various pH were diluted with absolute ethanol and deionized distilled water (DDW). The volume ratio of ethanol, cytochrome *c* solution with various pH, and DDW was 2:2:1. The LB trough (Series 2022, NIMA Tech., UK) was used to fabricate the cytochrome *c* LB films. DDW with a specific resistance of 18 Ω was used as a subphase. The temperature was constantly maintained at 297K. The extent of formation of cytochrome *c* LB films onto substrate was measured using UV-visible spectrophotometer (Jasco UV-550, JAPAN).

RESULTS AND DISCUSSION

In LB film formation, the cytochrome *c* molecules that spread onto subphase have gaseous state dynamics, and the molecules at the air-water interface were sensitively affected with pH variation.

As shown in Fig.1, π-A isotherms of cytochrome *c* LB films were obtained at various pH.

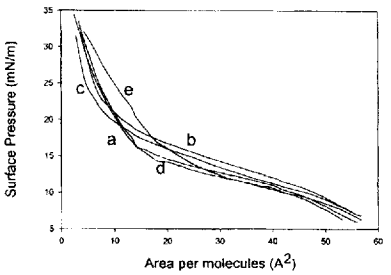

FIGURE 1. π-A Isotherms of cytochrome *c* ; pH 6.0(a); 7.0(b); 8.0(c); 9.0(d); 10.0(e)

From each π-A Isotherm, information on the stability of monolayer at the air/water interface, phase transitions, and conformational transformation can be obtained. In the area ranging from 12 to 40 $Å^2$/molecules, the liquid state of cytochrome *c* Langmuir monolayers was shown. Langmuir monolayers of cytochrome *c* exhibited a liquid-expand (LE) state above 34 $Å^2$/molecule, a liquid-condensed (LC) state at 34~10 $Å^2$/molecule, and a solid-like (SL) state below 10 $Å^2$/molecule. In the other pH conditions except pH 8, the curves gradually increased in the transition region from LC states to SL state. It is considered that some portions of cytochrome *c* molecules were dissolved into the subphase, or Langmuir multilayers were formed by compressing of the barrier. It was investigated by UV-visible spectroscopy that cytochrome *c* LB films deposited onto quartz substrate were fairly well formed. As shown in Fig. 2, the UV-Visible spectrum of cytochrome *c* LB films deposited onto the quartz substrate at various pH shows similar maximum absorbance peak. It represents that the cytochrome *c* molecules were not denatured significantly in all pH. However, the absorbance intensities of cytochrome *c* LB film onto quartz substrate were varied with pH and the maximum absorbance intensity at 410nm was observed at pH 8.0. The AFM image of cytochrome *c* LB film surface at pH 8.0 was shown in Filg. 3.

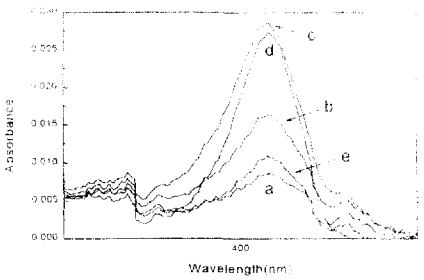

FIGURE 2. UV-visible absorption spectrum of cytochrome *c* LB films: a, pH 6.0; b, pH 7.0; c, pH 8.0; d, pH 9.0; e, pH 10.0

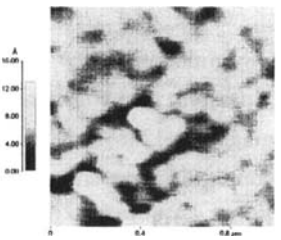

FIGURE 3. AFM images of cytochrome *c* LB film surface at pH 8.0

It can be found that the cytochrome *c* LB films at pH 8.0 showed much better deposition characteristics than the other conditions.

Acknowledgements

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References

[1] J. W. Choi, H.G. Choi, J. Min and W.H. Lee, Proc. of the 5th World Congress on Biosensors, **1**, 129 (1998).