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Fabrication of Cytochrome c Multi-Layers by Schaefer Technique

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Fabrication of Cytochrome *c* Multi-Layers by Schaefer Technique

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To spread cytochrome *c*, the water soluble protein, on subphase homogeneously, cytochrome *c* buffer solution was mixed with ethanol diluted with a deionized distilled water (DDW). After spreading it, cytochrome *c* multi-layers were deposited onto ITO coated glass by Schaefer technique (the horizontal lifting technique), and Langmuir-Blodgett (LB) technique (vertical lifting technique). Cytochrome *c* multi-layers deposited onto the substrate by Schaefer technique and LB technique were analyzed by UV-visible spectroscopy, and multi-layers of cytochrome *c* fabricated using the Schaefer technique was analyzed by Atomic Force Microscopy (AFM).

Keywords: Cytochrome *c;* Schaefer technique; Langmuir-Blodgett Film; Atomic force microscopy

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. The key feature of cytochrome *c*, the capability of electron transfer, is driven from the redox state change and conformational change of heme groups bound covalently via two thioether linkages formed by two cysteine side chains and two axial ligands, histidine and methionine. Since cytochrome *c* is acting as an electron acceptor 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. For these characteristics of cytochrome *c*, the fabrication of cytochrome *c* films is considered as the most important process in the development of bioelectronic device[1]. But, because cytochrome *c* is a water-soluble protein, it is difficult to directly fabricate the molecular multi-layers onto substrate[2].

The objective of this research is to fabricate cytochrome *c* multilayers onto substrate by the Schaefer technique[3] (a horizontal lifting technique), comparing with LB technique (a vertical lifting technique). It has not yet been reported that cytochrome *c* multilayers onto substrate by the Schaefer technique can be fabricated.

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EXPERIMENTAL DETAILS

Cytochrome *c* from horse heart (type IV, Sigma Chemical Co.) was used. Cytochrome *c* was mixed with phosphate buffer solution with pH 8. Its concentration is 4.0μM. For spreading cytochrome *c* solution on the subphase, cytochrome *c* was mixed with ethanol diluted with deionized distillrd water (DDW). The volume ratio of ethanol, cytochrome *c* and DDW is 2:2:1. To fabricate cytochrome *c* multilayers onto the substrate, an LB trough (Series 2022, NIMA) was used. DDW with a specific resistance of 18MΩ after distillation was used as a subphase.

RESULTS AND DISCUSSION

Fig. 1 shows the surface pressure-area isotherm of cytochrome *c* spread on the subphase. From the surface pressure-area isotherm, information on the stability of monolayer at the water-air interface, phase transitions and conformational transformation are obtained. As shown in Fig. 1, a phase transition is shown at 18 mN/m. Thus cytochrome *c* multilayer fabrication onto the substrate was carried out at 20mN/m.

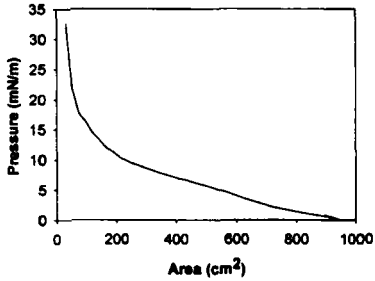

FIGURE 1. π-A Isotherm of cytochrome *c* (cm^2: Area of trough)

It was investigated by UV-VIS spectroscopy that cytochrome *c* multilayers deposited onto substrate were formed well. As shown in Fig. 2, the maximum absorbance peak of cytochrome *c* was about 410 nm and the absorbance intensity of cytochrome *c* thin films deposited onto substrate increased approximately proportional to its stroke number by Schaefer technique and LB technique. Also, the absorbance intensity (i.e. molecular density of cytochrome *c*) of the films by Schaefer technique is higher than that by LB technique. Based on UV-vis absorbance, it could be suggested that to form the cyt *c* films the Schaefer technique was more suitable than the LB technique. This may occur because the cytochrome *c* was removed by subphase at down stroke in the LB technique.

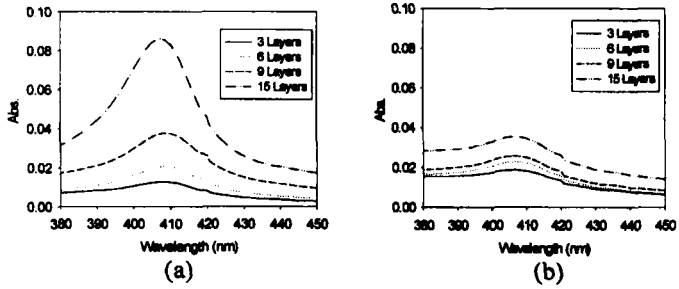

FIGURE 2. UV-vis absorbance as cytochrome *c* layer (a) by Schaefer technique and (b) by LB technique

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The morphology of cytochrome *c* multi-layers onto ITO-coated glass by Schaefer technique was observed using the AFM. As shown in Fig. 3, the height of cytochrome *c* films is about 150Å and the size of protein aggregates is about 0.5μm. Based on these results, cytochrome *c* multi-layers were well formed onto substrate.

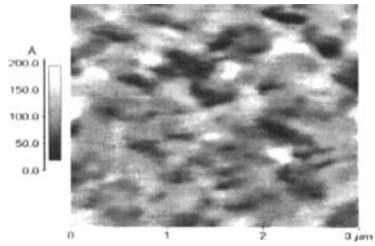

FIGURE 3. Morphology of cytochrome *c* by the AFM (3-layers).

It could be observed that cytochrome *c* multi-layers deposited onto substrate were well formed by Schaefer technique based on UV-VIS absorbance and AFM image. Thus Schaefer technique seems feasible for fabricating multilayers of cytochrome *c*, which opens the way to fabricate molecular bioelectronic device consisting of cytochrome *c*.

Acknowledgement

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