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For the development of preferable immunosensor and protein chip, protein A molecules thin film to have specific affinity to the Fc protion of immunoglobulin G (IgG) was fabricated using Langmuir-Blodgett (LB) technique. The existence of immobilized IgG on the protein A LB film was verified by using fluorescence marker and the fluorescence spectroscopy. The surface morphologies of the protein A LB film and adsorbed IgG layer on the protein A LB film were analyzed by using Atomic Force Microscopy (AFM).

Keywords: Langmuir-Blodgett Technique, Protein A LB film, Protein Chip, Immunoglobulin G,

INTRODUCTION

Antibody-based immunoassays such as immunosensors and protein chip are the most commonly used type of diagnostic assay and still one of the fastest growing technologies for the analysis of biomolecules [1]. The choice of antibody immobilization method in antibody-based immunoassays is very important because it is a crucial to decide the orientation of antibody molecule on the solid substrate and the binding characteristics between antibody and antigen. For the development of immunosensor and protein chip with high performance, F_{ab} fragment of IgG should be posed in opposition to the solid substrate. Protein A, a

cell wall protein of *Staphylococcus aureus* (M.W. ca. 42,000), has specific affinity to the Fc portion of IgG. Molecular membrane of protein A is regarded as a versatile supporter for immobilization of an antibody molecule. Langmuir-Blodgett (LB) technology is known as a useful method for the formation of well-oriented film of bio/organic material on a solid substrate, because it can control the degree of order and packing density of protein, and especially minimize the loss of protein activity. The objective of this research is to fabricate the protein A LB film in order to achieve highly oriented IgG layer. It is indispensable for the production of highly sensitive immunosensor or protein chip. The proposed technology will give us a way to fabricate the immunosensor or protein chip

EXPERIMENTAL DETAILS

All chemicals used in this study were obtained commercially as the reagent grade. In order to prepare a hydrophobic surface of a substrate, after rinsed, it was dipped in 0.2% n-octadecyltrichlorosilane solution for 30min and then rinsed with fresh toluene[2]. Protein A LB film was deposited on the pretreated substrate with circular trough (NIMA, England). 1mM N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES) buffer of pH 7.0 was used as a subphase. Arachidic acid methyl ester and trimethylstearylammonium chloride mixture (molar ratio 4:1) were used as lipids for protein A LB film fabrication[2]. The mixed lipid was spread on subphase for 20min and then compressed until the surface pressure would reach to 20mN/m. After 5min, 2.5mg/ml protein A solution was injected into the subphase for protein A to be adsorbed on the spread lipid layer. Adsorption of protein A was completed for 1hr, which depended on the electrical charge difference between protein A and lipids. And then, protein A lipid monolayer was expanded until surface pressure would be 10 mN/m in order that the adsorbed protein A was among the lipids. Finally, the expanded monolayer was recompressed to the 20mN/m of surface pressure. Under the condition, protein A LB film is fabricatied. The surface morphologies of the protein A LB film and adsorbed IgG layer on the protein A LB film were analyzed by using AFM (Auto Probe, CP, PSI, U.S.A). The IgG was labeled using fluorescein isothiocyanate (FITC) and immobilized on the protein A LB film. The existence of immobilized IgG on the protein A LB film was verified by using the fluorescence spectroscopy (Spectrapro 300i, Acton inc., U.S.A).

RESULTS AND DISCUSSION

π-A curves for pure lipid monolayer and protein A-lipid layer were obtained through the compression and release of surface area. Monolayer of protein A molecules was expected to be formed by lipid layer having positive charge in buffer solution at pH 7.0 because of negative charge of protein A. The formation of lipid protein A monolayer was experimentally proved from the π-A curve as shown in Fig. 1. The π-A curve for lipid monolayer after protein A adsorption was shifted in the range of 30-80cm^2 in comparison with that of lipid monolayer before protein A adsorption. It was thought that the shifting of π-A curve for lipid layer after protein A adsorption would result from the increase of volume of a molecule per area. In other words, the increase of surface pressure meant that the volume of a molecule occupying the constant area should be increased by protein A adsorption. Therefore, it can be concluded that the protein A monolayer was formed by lipid monolayer and could be deposited onto a hydrophobic substrate.

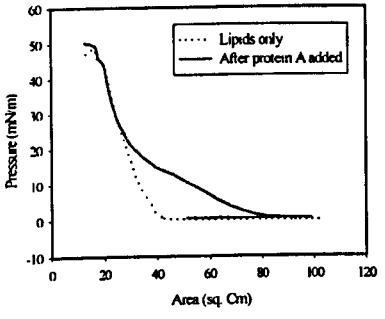


Fig. 1 π-A Curve of lipid layer and protein A lipid layer

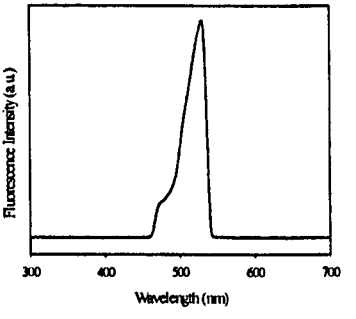
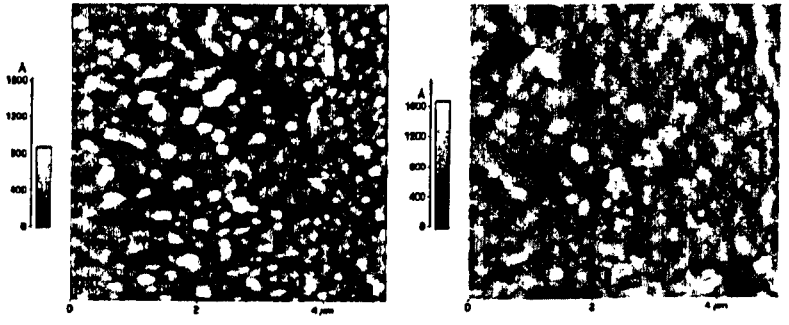


Fig. 2. Fluorescence intensity profile FITC-labeled IgG layer adsorbed on the protein A LB film

In order to verity the existence of IgG on the protein A LB film, fluorescence spectrophotometry was applied to the deposited FITC-labeled IgG layer. Fig. 2 shows the fluorescence emission peak when the light at the wavelength of 465 nm put into the adsorbed IgG

layer on the protein A LB film. The maximum peak was observed at the wavelength of 535 nm, which means the IgG layer was successfully adsorbed on the protein A LB film.

Fig. 3 shows the surface topographies of protein A LB film and IgG layer deposited on the protein A LB film by AFM. Compared with the protein A LB film, the height increase was observed in the IgG layer, which means the IgG layer was successfully fabricated. In Fig. 3(b), IgG molecules were adsorbed onto the protein A LB film as an aggregated pattern in solid-like state with keeping its random cloud-like structure as in bulk solution.



(a) (b)

Fig. 3 AFM Tolographies of (a) protein A LB film, (b) adsorbed IgG layer on the protein A LB film

From the above results, it can be concluded that IgG layer was fabricated using protein A LB film.

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