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Fabrication of Protein A-Viologen Hetero LB Film for Antibody Immobilization

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Abstract

For the development of preferable immunosensor and protein chip, the viologen Langmuir-Blodgett (LB) multilayer was fabricated on the surface, and then protein A was adsorbed on the proposed viologen LB film by electrostatic attractive force. The Immunoglobulin G (IgG) labeled with fluorescence marker was self-assembled on the fabricated protein A film. The topographies of the deposited films were investigated by using atomic force microscope (AFM). The immobilization of IgG was verified by fluorescence spectrum. Such structures can be used as sublayers for various kinds of IgG immobilization toward immunosensors and protein chip.

Introduction

Antibody-based immunoassays are the most commonly used type of diagnostic assay and still one of the fastest growing technologies for the analysis of biomolecules[1]. Control of antibody configuration become very important as the requirements for the antibody sensitivity increase. In order to enhance the sensitivity it is necessary to bind antibodies in a well-designed configuration. Protein A, a cell wall protein of *Staphylococcus aureus* (M.W. ca. 42,000), has specific affinity to the Fc portion of immunoglobulin G (IgG). One of the most important points in the design of an immunoassay or protein chip is the choice of the immobilization method. 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[2,3], because it can fabricate well-ordered thin films and control the degree of order and packing density of protein which is indispensable for the production of highly sensitive immunosensor or protein chip. This research will propsose a way to produce many immunosensors and protein chips.

Materials and Methods

Glass substrates were immersed in piranha solution and washed several times with deionized distilled water before use. *Staphylococcusal* protein A (SpA) and phosphate buffer saline (PBS) was purchased from Sigma-Aldrich U.S.A., respectively. Viologen dissolved into chloroform was spread onto subphase and deposited using the circular-type Langmuir-Blodgett trough(Model No. 2011, Nima Tech, UK) at room temperature. The fabricated viologen LB film was immersed into the SpA solution at pH 7.4. The fabricated viologen-SpA hetero-film was investigated using UV-vis spectrophotometry (Jasco UV-550, JAPAN). The AFM images were obtained with Auto Probe (CP, PSI, U.S.A.) at room temperature in air[4]. Human immunoglobulin G (IgG, reagent gr., Sigma-Aldrich, U.S.A.) was labeled with fluorescein isothiocyanate (FITC)[5]. The unlabeled FITC was separated by using Sephadex G-25 gel-filtration column. The IgG bound on the protein A hetero-film was investigated with fluorescence spectrophotometric system equipped with SpectraPro 300i (Acton Research Corp., MA, U.S.A.)

Results and Discusstion

For the production of well-oriented viologen film, π-A curve for viologen monolayer was investigated through the compression of surface area. Fig 1 shows the π-A curve for viologen monolayer which gives the information on the stability of monolayer at the air-water interface. Phase transition and conformational transformation were observed around the surface pressure of 40 mN/m. The formation of viologen monolayer was experimentally proved from the π-A curve. Thus the viologen LB film onto the substrate was fabricated at the surface pressure of 40 mN/m.

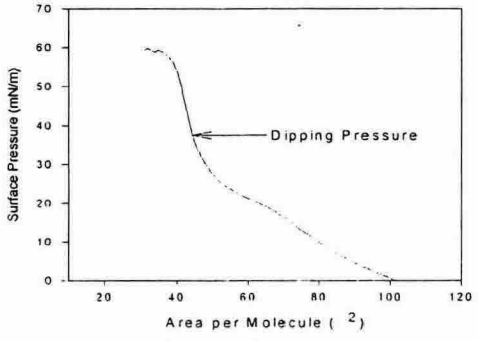

Fig. 1 the π-A curve for viologen monolayer

AFM topographies verified the formation of Protein A and IgG film. Fig. 2 shows the topographies of viologen LB film, viologen-protein A hetero-film, and IgG layer immobilized on protein A hetero-film. The heights of protein A and IgG were about 60.0 Å and 150.0 Å, respectively. The height increase was observed in the IgG layer, which means the IgG was successfully fabricated. From those results, the protein A and IgG were well formed onto the substrate.

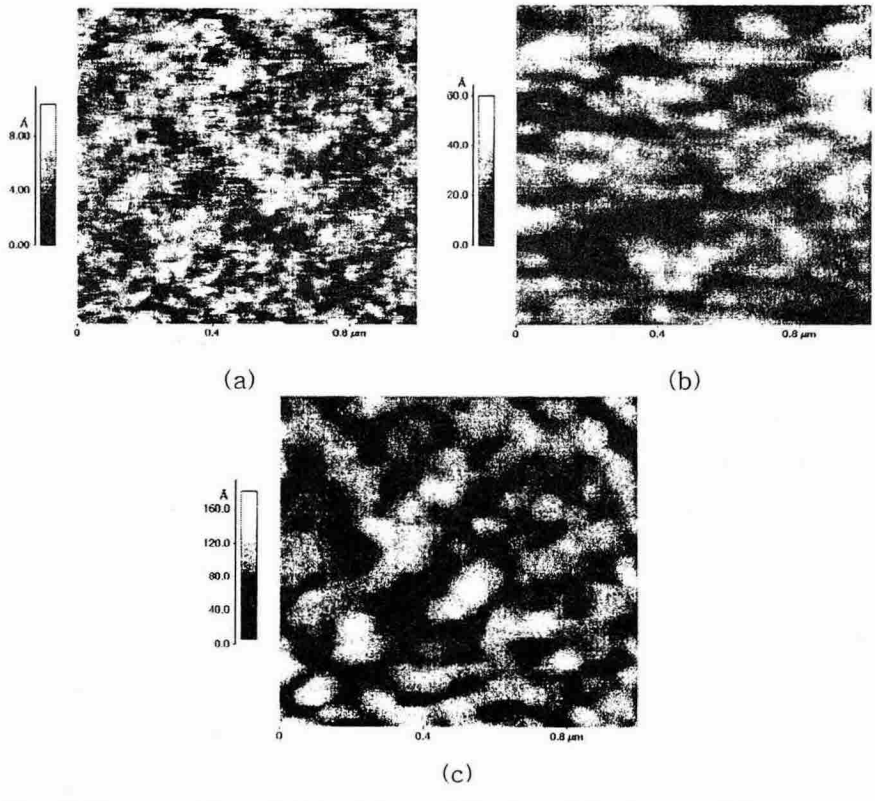

Fig. 2 Topographies of (a) viologen LB film, (b) viologen LB-protein A hetero-film, and (c) IgG immobilized on the protein A hetero-film by AFM

The IgG labeled with FITC was observed through the fluorescence spectrophotometry. The labeled FITC is excited by absorbing light at the wavelength of 495 nm, and then emits the light at the wavelength of 525 nm. Fig. 3 shows the fluorescence spectrum of the FITC labeled IgG immobilized on the viologen-protein A hetero-film. The proposed fluorescence spectrum shows that the maximum peak is observed near the wavelength of 525 nm, which

means the existence of FITC-labeled IgG.

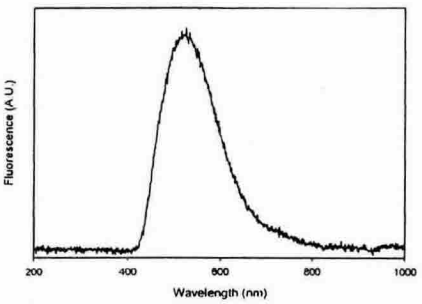

Fig. 3 Fluorescence spectrum of FITC labeled IgG immobilized on the viologen-protein A hetero-film.

The proposed technology can pave the way for the production of the antibody-based protein chip in biomedical research and clinical chemistry.

Acknowldegement

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References

1. A. Barraud, H. Perrot, V. Billard, C. Martelet, & J, Therasse, "Study of immunoglubulin G thin layers obtained by the Langmuit-Blodgett method: application to immunosensors"(1993), Biosensors & Bioelectronics, **8**, 39-48

2. A. Ulman, "An Introduction to ultrathin organic films from Langmuir-Blodgett to Self-Assembly"(1991), Academic Press,· 127.

3. J.-W. Choi, J. Min, J.-W. Jung, H.-W. Rhee, and W.H. Lee, "Fiber-optic biosensor for the detection of organophosphorus compounds using the AChE-immobilized viologen LB films"(1998), Thin Solid Films, **327-329**, 676-680

4. S.N. Magnov, M.-H. Whangbo, "Surface Analysis with STM and AFM" (1996), VCH Publishers, Inc., NY, USA, 243-244.

5. E. Harlow and D. Lane, "Antibodies: A laboratory manual"(1988), Cold Spring Harbor, NY, 353-355.