표면플라즈몬 공명현상을 이용한 *Escherichia Coli O157:H7* 검출용 면역센서

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Immunosensor for the Detection of *Escherichia Coli O157:H7* Using Surface Plasmon Resonance

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INTRODUCTION

Escherichia coli O157:H7 is a major food-borne pathogen in humans and is causing increasing concern world-widely as the incidence continues to rise [1]. The major impact of the bacterial induced diarrhea disease is on children under the age of 10 and on elderly people living in less-developed countries, in which bacterial diarrhea diseases remain a significant public-health problem.

Recently, the immunosensor based on surface plasmon resonance (SPR) have been developed for measurement of antigens binding to antibody molecules immobilized on the SPR sensor surface, which are capable of detecting analytes in complex biological media with high specificity and sensitivity, with short detection time, and with simplicity [2,3]. However, few researches of immunosensors for the detection of *E. coli O157:H7* based on SPR have been reported.

The objective of this study is to develop the immunosensor for detection of *E. coli O157:H7* based on SPR. In order to endow the orientation of antibody molecules on SPR sensor surface, self-assembled protein G layer on Au substrate was fabricated. For minimizing the steric hindrance by antigen size in the binding characteristic between antibody and antigen, 2D configuration of immobilized antibody molecules on solid surface was controlled by molar ratio variation between 11-(MUA) and hexanethiol. The formation of self-assembled protein G layer on Au substrate, and the binding of antibody and antigen in series were confirmed by SPR spectroscopy. The surface morphology analyses of self-assembled protein G layer on Au substrate, monoclonal antibody (Mab) against *E. coli O157:H7* immobilized on self-assembled protein G layer and bound *E. coli O157:H7* extracts on immobilized Mab against *E. coli O157:H7* were performed by atomic force microscope (AFM).

EXPERIMENTALS

Protein G (M.W. 22600 daltons) was purchased from Prozyme Inc. (USA). *E. coli*

O157:H7 (ATCC 43895) was kindly offered from American Type Culture Collection (USA). Mab against *E. coli O157:H7* was obtained from Fitzgerald Industries International, Inc. (USA). Other chemicals used in this study were obtained commercially as the reagent grade.

BK 7 glass plate (18 mm × 18 mm, Superior, Germany) was used as the solid support and Au was sputtered to the BK 7 glass surface. The Au surface was cleaned using pirahna solution (30 vol.% H_2O_2 and 70 vol.% H_2SO_4) at 60°C for 5minutes, and then rinsed with ethanol and deionized water. Thin layer of 11-mercaptoundecanoic acid (MUA) on Au surface was prepared by submerging Au substrate into a glycerol/ethanol (1:1, v/v) solution containing 150 mM 11-(MUA) for at least 12 hours [4]. For chemical binding between 11-(MUA) adsorbed on Au and free amine of protein G, the carboxyl group of 11-(MUA) was activated by submerging Au substrate modified with 11-(MUA) into a solution of 10% 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDAC) in water/ethanol (10/1, v/v) for 2h at room temperature. Self-assembled protein G layer was fabricated by the incubation of the activated Au substrate in a solution of 10 mg/L protein G in 10mM phosphate buffer (pH 7.4) containing 0.14 mol/L NaCl and 0.02% (w/v) thimerosal (PBS) at room temperature for 2h. For the immobilization of antibody, a solution containing antibodies (50 pmol/mL Mab against *E. coli O157:H7*) in PBS buffer was applied to the self-assembled protein G layer. After 2h incubation, the surface was washed with PBS buffer and incubated for 20 min with PBS containing 0.1% Tween 20, followed by washing with PBS buffer. For minimizing the steric hindrance by antigen size in the binding characteristic between antibody and antigen, 2D configuration of immobilized antibody molecules on solid surface was controlled by molar ratio variation between 11- (MUA) and hexanethiol in the range of 1:0 to 1:5. The formation of self-assembled protein G layer on Au substrate, and the binding of antibody and antigen in series were confirmed by SPR spectroscopy. The surface morphology analyses of self-assembled protein G layer on Au substrate, Mab against *E. coli O157:H7* immobilized on self-assembled protein G layer and bound *E. coli O157:H7* extracts on immobilized Mab against *E. coli O157:H7* were performed by AFM

RESULTS AND DISCUSSION

The changes of SPR curve by adsorbing 150mM 11-(MUA), and by chemical binding of protein G in series on Au substrate, by adsorbing Mab against *E. coli O157:H7* (50pmol/mL Mab against *E. coli O157:H7*) on self-assembled protein G layer, by formation of immobilized Mab against *E. coli O157:H7* - *E. coli O157:H7* extracts complex were shown in Fig. 1. As a result, the SPR minimum position was shifted significantly from 43.002°±0.03 to 43.257°±0.04 by the adsorption of 150mM 11-(MUA) on Au surface. And, the SPR minimum position was shifted from 43.257°±0.04 to 43.437°±0.03 by chemical binding between protein G and the activated carboxyl group of 11-(MUA) with EDAC. The shift in the SPR minimum position verified that thin layer of 11-(MUA) on Au surface was formed and protein G molecules were well bound with 11-(MUA) adsorbed on Au substrate.

And, the SPR minimum position was shifted significantly from 43.437°±0.03 to 43.607°±0.03 by the binding of Mab against *E. coli O157:H7* on self-assembled protein G layer, and SPR minimum position was shifted from 43.607°±0.03 to 43.907°±0.05 by formation of immobilized Mab against *E. coli O157:H7* - *E. coli O157:H7* extracts complex, since a shift of SPR minimum position resulted from the adsorption of dielectric materials on

SPR sensor surface.

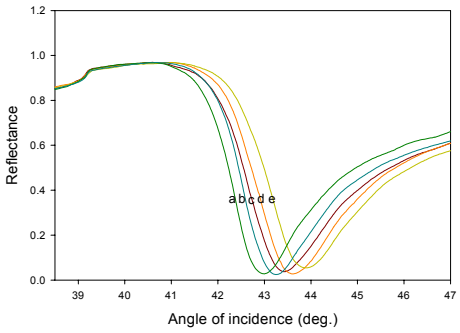

Fig. 1. The changes of SPR curve by adsorbing from 11-(MUA) to *E.coliO157:H7* in series. (Lines; a: bare gold, b: 150mM 11-(MUA), c: protein G, d: Mab against *E.coli O157:H7*, e: *E.coliO157:H7*).

The changes of SPR minimum position shift by binding between immobilized Mab against *E.coliO157:H7* on self-assembled protein G layer with various molar ratio of 11-(MUA) and hexanethiol and *E.coliO157:H7* extracts were shown in Fig. 2.

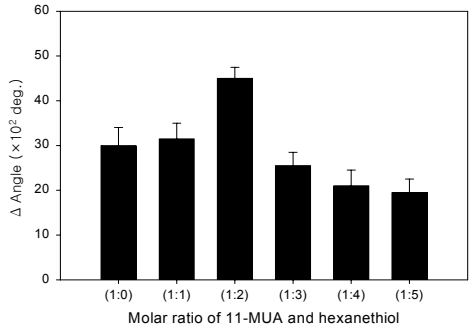

Fig. 2. The changes of SPR minimum position shift by binding between immobilized Mab against *E.coliO157:H7* on self-assembled protein G layer with various molar ratio of 11-(MUA) and hexanethiol and *E.coliO157:H7* extracts;

The optimal molar ratio of 11-(MUA) to hexanethiol for the formation of Mab against *E.coliO157:H7* *E.coliO157:H7* extracts complex is 1 to 2 (50 mM 11-(MUA) to 100 mM hexanethiol). Ideally, binding the antibody in exposing the paratope is favored by dense packing of antibody molecules on the SPR surface. Dense packing by itself, however, is not sufficient for optimal antibody antigen complex formation. Antigens need lateral access to the antibody paratope for unimpaired binding and a larger antigen needs more space between antibodies to access the paratope than a smaller antigen. That is, the effect of the steric hindrance by antigen size in the binding characteristics between antibody and antigen must be minimized for developing SPR immunosensor with high efficiency.

The degree of SPR minimum position shift by binding between immobilized Mab against *E. coli O157:H7* and various concentrations of *E. coli O157:H7* (LPS) were shown in

Fig. 3.

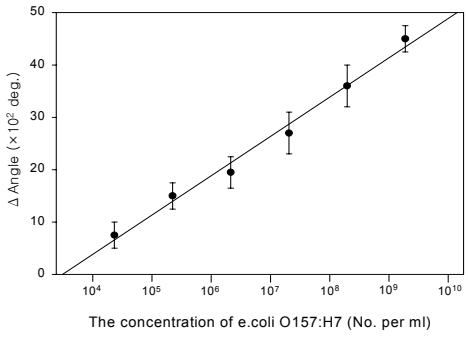

Fig. 3. The changes of SPR minimum position shift by binding of various concentrations of *E.coliO157:H7* extracts.

As shown in Fig. 3, the shift degree of SPR minimum position also increases linearly, as the concentration of cells increases. The lowest detection limit of the immunosensor based on SPR was 10^4cells/mL and the assay is two orders of magnitude more sensitive than a standard ELISA.

CONCLUSION

Immunosensor using SPR onto self-assembled protein G layer was developed for the detection of *E. coli O157:H7* and its lower detection limit was 10^4 cells per ml. The fabrication technique of SPR sensor used in this study for the detection of *E. coli O157:H7* could be applied to construct the immnosensors or protein chip with high efficiency.

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