Signal Amplification of Surface Plasmon Resonance based on Gold Nanoparticle-Antibody Conjugate and its Application to Protein Array

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ABSTRACT

Surface plasmon resonance (SPR) is a versatile, convenient, and low-power technique that may be used to determine the change of thin dielectric film. Although the sensitivity is very high, when small molecule is a target for immobilization or detection, the changes in the angle-dependent attenuated total reflectance (ATR) are often small so the use of SPR method is limited for generic application. The specific interaction between target molecules and Au nanoparticle-antibody conjugate induce the change of the surface properties such as mass and electric coupling, which can be represented as the change of plasmon resonance angle. In this study, gold (Au) nanoparticle-antibody conjugate was applied for the signal enhancement of SPR. Antibody was immobilized with an oriented configuration on Au surface. Au nanoparticle-antibody conjugated was prepared after coagulation test based on spectrophotometric method. Experimental results showed that tremendous signal amplification was observed, which represent remarkable advances of SPR technique for ultrasensitive detection. And the detection limit of binding events was also improved.

Keywords: surface plasmon resonance, nanoparticle, antibody immobilization, signal amplification

1 INTRODUCTION

Surface plasmon resonance (SPR) is a versatile technique to probe and characterize physical or chemical changes on thin films. Because the sensitivity of SPR is superior to traditional technique where binding event signal transduction is difficult or impossible to accomplish by traditional optical spectroscopies, it has been widely studied for the measurement of protein-protein interaction [1]. The vast majority of papers on biological SPR describe experiments conducted on commercial instrumentation that utilize an extended coupling matrix to decrease nonspecific binding and to increase surface loading of biomolecules [2]. Protein binding events leading to small changes in the refractive index of the fabricated biosurface are detected via corresponding small changes in the angle-dependent attenuated total reflectance (ATR).

However, the use of the SPR method is hindered by the fact that the changes in the refractive index as a result of binding processes are often small, and so the systems display limited sensitivities [3]. Method for the enhancement of the changes in the refractive index at the sensing surface can be implemented through the development of hybridized material which is both specific to target material and strong optical coupling with metal film. Au nanoparticle can be a competent candidate for detection of SPR enhancement. The possibility of synthesizing metal nanoparticles of controlled size and surface capping allows the generation of low molecular weight- or biomaterial- Au nanoparticle conjugates. When the nanoparticles are captured on the biosurfaces, the nanoparticles are expected to work as the electronic couping between the localized surface plasmons created by p-polarized laser. Subsequently, significant shift of plasmon resonance angle can be occurred on the fabricated biosurface.

Recently, a new analytical technique based on metal and magnetic nanoparticles have been developed [4]. Nanoparticles composed of gold, silver, and copper have intrinsic property to adsorb the protein, which can be applied to the detection of specific protein-protein interaction (both the specific binding of target protein and detection simultaneously). Because the size of nanoparticle is ranged from a few to several tens, the nanoparticle-protein complex has been utilized for a number of applications such as immunochromatography, conductance measurement, and surface enhanced-Raman scattering and so on [5].

In this study, for the construction of ultrasensitive biosensing device, application of Au nanoparticle-antibody conjugate is carried out for signal enhancement of surface plasmon resonance. For preparing stable and constant-sized Au nanoparticle-antibody conjugate, the optimal condition for the fabrication of gold nanoparticle-protein complex was investigated through the coagulation test, UV/vis. spectroscopy. Through the SPR, biosurface fabrication and detection of target molecules were investigated respectively. The proposed technique can enhance the detection limit compared to the label-free SPR analysis.

2 MATERIALS & METHODS

2.1 Materials and preparation of gold film.

All solutions were prepared with Millipore (Milli-Q) water. Monoclonal and polyclonal antibody (Mab, Pab) against human serum albumin were purchased from Sigma (St. Louis, MO, USA). Human serum albumin (HSA), 2-mercaptoethylamine (MEA), and Gold (Au) nanoparticle with a mean diameter of 5 nm were also purchased from Sigma. Other chemicals used in this study were obtained as reagent grade. Au film with a thickness of 43 nm was purchase from Inostek Inc., (Korea). The prepared Au surface was cleaned with piranha solution before thin film fabrication.

2.2 Fabrication of self-assembled layer with antibody fragment on Au surface

To prevent the enlargement of nanoparticle-antibody complex and endow the orientation of antibody, native thiol group in the antibody molecules was applied for the construction of nanoparticle-antibody complex. 10 mg of 2-MEA was applied to the 1 mL of antibody solution (up to 10 mg/mL) for the fragmentation of IgG molecules. The reaction was carried out for 90 minutes under 37℃. After reaction, the residual 2-MEA was removed through the dialysis against PBS-EDTA buffer with pH 7.4 which was composed of sodium phosphate, EDTA.

2.3 Formation and characterization of nanoparticle-antibody conjugate

For the fabrication of Au nanoparticle-antibody conjugate, coagulation test was carried out ahead in order to find out the optimal concentration of antibody solution which minimizes the disturbance of the nanoparticle-antibody complex In this study, mixed solution of Au nanoparticle and antibody fragment was prepared after dilution with water [6]. The prepared solution was incubated for 2 hr at 4 ℃. For the stabilization of Au nanoparticle-antibody conjugate, 0.1 ml of 5% casein was added in the mixed solution and it was incubated for 1 hr at 4 ℃. The prepared Au nanoparticle-antibody conjugated was centrifuged at 34,000 rpm for 1 hr. at 4 ℃ in Beckman ultracentrifuge (Optima XL-90, Beckman Instruments, Inc., CA, USA) equipped with a SW50.1 swing bucket rotor. After centrifugation, the sediments corresponding to Au nanoparticle-antibody complexes were recovered and resuspended in 0.5 mL of PBS.

2.4 Investigation of biomolecular interaction and surface analysis

The measurements of SPR (Multiskop™, Optrel Inc., Germany) were done to verify the formation of antibody surface and antibody-antigen interaction. The instrumental configuration of the laser light source, polarizer, photo-multiplier tube, and attenuated total reflection (ATR) coupler were the same as in the cited reference [7]. In order to estimate the thickness of the fabricated antibody fragment, the ellipsometric angles (del, psi) of the layers fabricated with different concentrations were measured with the traditional null-ellipsometry system which is combined with SPR. The topographic observation of the prepared biosurface was carried out with atomic force microscopy (AFM, Autoprobe CP, Park Scientific Instruments, USA).

3 RESULTS AND DISCUSSION

Fig. 1 shows the SPR spectroscopy of the prepared antibody fragment (half fragment), antigen, and Au-nanoparticle antibody conjugate, respectively. At the SPR angle of bare gold surface, the SPR minimum angle was forwardly shifted in a degree of 0.5 approximately. After the blocking procedure with 3 % (w/w) of BSA, the HSA (antigen) was applied to the fabricated antibody surface, which induced the forward shift of plasmon angle. When the Au nanoparticle-antibody conjugate was applied to the surface, the plasmon angle shift was occurred significantly, which was due to the specific interaction between the Au nanoparticle and evanescent wave generated by surface plasmon. Compared with the SPR angle shift generated by label-free type SPR system, the proposed technique was expected to lead to enhanced shift of the resonance angle.

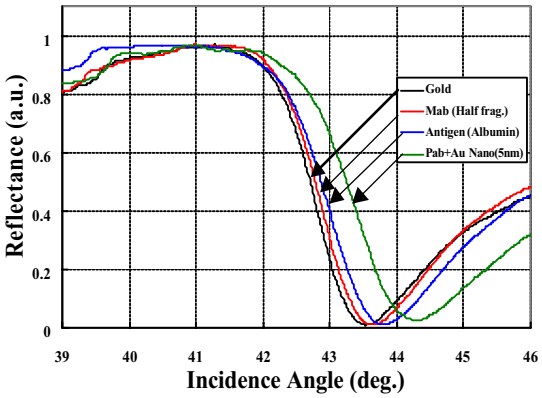
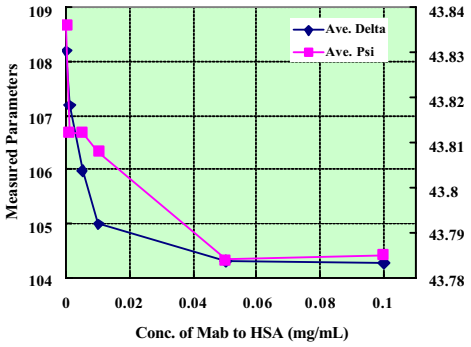


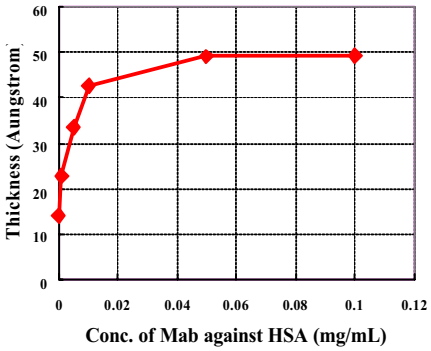
Fig. 1. SPR spectroscopies with respect to the film fabrication step on the prepared Au surface

Fig. 2 shows the change of the del and psi with respect to the each layer. In the thin film model, the adsorption of a protein onto solid surface results in the change of del and the change of psi is dependent on the nature of the substrate [8]. With the three-phase optiocal model (ambient-film-gold), the thickness values of the protein layers were calculated by means of the software designed for elipsometric analysis obtained from Optrel Gbr. The refractive index of the gold layer in the optical model was assumed to be 0.183 + j3.090 [9]. As shown in the Fig. 2,

the acquired del and psi values were monotonically decreased as the concentration of Mab HSA half fragment increased. In accordance with the variation trend of the measured ellipsometric angles, the film thickness was monotonically increased with respect to the concentration of Mab HSA half fragment. The film thickness was not changed any more at the 0.05 mg/mL of Mab HSA half fragment. Because the standard deviation was not more than 0.3 Å of the calculated thickness, the fabricated thin film showed regular configuration on the overall surface.



(a)



(b)

Fig. 2. Ellipsometry study of the fabricated film with respect to the concentration of antibody; (a) del-psi relationship and (b) calculated film thickness as the concentration increases

As mentioned above, Au nanoparticles have an intrinsic property to absorb visible light at a wavelength of 525 nm (A_{525}). Also, proteins have A_{280} though their absorption is not strictly quantitative [10]. Because the value of A_{525} has a linear relationship with regards to the nanoparticle content, the comparison between A_{525} and A_{280} help us to confirm the existence of antibody conjugated on the Au nanoparticle surface. The mixed Au nanoparticle-antibody conjugate and the residual antibody were separated through ultracentrifuge and the supernatant (antibody residue) was excluded from the pure conjugate. Fig. 3 shows the absorbance values with a form of bar graphs of pure Au nanoparticles and Au nanoparticle-antibody conjugate. The comparison of the values of A_{280} may lead to errors because the amount of nanoparticle was not considered. A_{280}/A_{525} value was introduced for correct comparison. The value of A_{280}/A_{525} of pure Au nanoparticle was significantly less that that of the nanoparticle-antibody conjugates as shown in Fig. 3. From the experiment results above, the existence of nanoparticle-antibody conjugate was verified from the analysis of UV/vis. spectra.

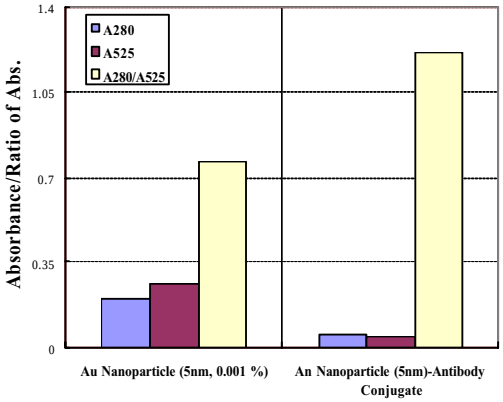


Fig. 3. Comparative study for the formation of gold nanoparticle-antibody conjugate. Because the A_{525} has a perfect correlation with respect to concentration, normalization is possible to compare the control.

Since the diameter of the fabricated nanoparticle was about 20 nm, the nanoparticle-antibody conjugate was observed as a form of dot in the overall scan range. An abrupt topographic change could be observed near the region expressed as the dot. Although the size of nanoparticle was somewhat distributed in the overall region, it was relative regular in the overall region. When 1 ng/mL of HSA was applied to the surface, the topography of the fabricated thin film was shown like Fig. 4, which means the lower concentration of antigen can be detected based on the proposed system.

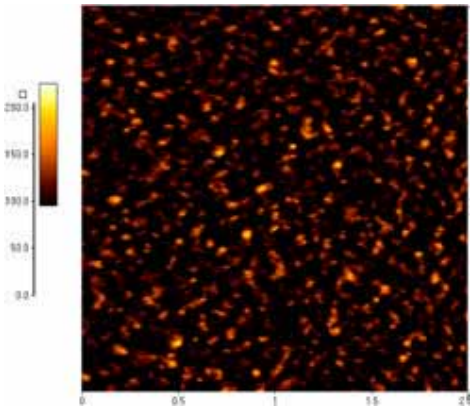


Fig. 4. AFM topography of the fabricated gold nanoparticle-antibody complex.

Fig. 5 shows the SPR spectroscopy of the fabricated thin films. When 100 ng/mL of HSA was applied to the fabricated biosurface, 0.111 degree of the plasmon resonance angle was shifted on the fabricated biosurfaces. Subsequent addition of the prepared nanoparticle-antibody conjugate made the plasmon resonance angle shifted forwardly in a degree of 0.52°.

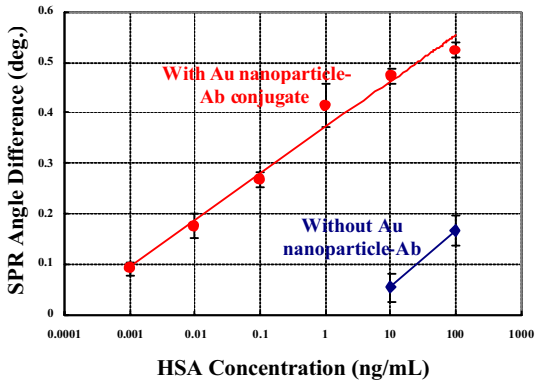


Fig. 5. Plot of the SPR angle difference vs. antigen (human serum albumin, HSA) concentration

As mentioned in the introduction, the signal enhancement of SPR was caused by nanoparticles. Compared with label free detection of HSA, there was much angle difference which suggested the extension of detection limit. When the concentration of target protein decreased, the amount of the nanoparticle-antibody conjugate bound on the fabricated biosurface was expected to decrease on the fabricated surface. Fig. 5 shows the plot of SPR angle difference versus HSA concentration. As the concentration of HSA decreased, the angle difference occurred by the enhanced SPR also decreased. Although a little deviation from the linear trend was observed in the presented plot, linear relationship was achieved in the designated region. The detection limit was 0.0001 ng/mL of HSA, which means that the designed assay system showed the 10^5 fold enhancement of sensitivity.

4 CONCLUSION

In the SPR measurement system, the signal enhancement could be achieved through the introduction of Au nanoparticle. In this study, Au nanoparticle-antibody conjugate was fabricated with the antibody fragment. Through the spectrophotometric studies, the conjugation between Au nanoparticle and antibody fragment was successfully carried out in the oriented manner. The optimal fabrication condition (concentration and incubation time) was determined for the constant size of the fabricated nanoparticle-antibody conjugate. The AFM topography showed that an abrupt height change due to nanoparticle-antibody conjugate existed and the detection of low concentration below 1.0 ng/mL is possible. Through the plot of SPR angle difference vs. antigen concentration, the linear correlation was achieved, of which the detection limit was 100 fg/mL.

ACKNOWLEDGEMENT

This research was supported from The Eco-technopia 21 project (2004-10002-0019-0) of the Ministry of Environment of Korea.

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