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Surface Plasmon Resonance Imaging Detection of DNA Hybridization Using Colloidal Au Attached Probe DNA

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Surface plasmon resonance (SPR) spectroscopy is one of simple methods for detection of a DNA hybridization adsorption. Since the SPR angle (angle of incidence at minimum reflectivity) shifts of single-stranded DNA monolayer on a metal surface is induced by the duplex formation, this method allows label-free and in-situ detection of DNA hybridization adsorption. However, the SPR angle shift for the hybridization adsorption is small and the sensitivity of the SPR method is low. Our previous work demonstrated that the colloidal Au attached probe DNA monolayers could enhance the SPR angle shift (about a 3.5 times larger compared with non-attached probe DNA) without any labeling of target DNA under atmospheric condition. In this work, we examined the SPR response of DNA hybridization adsorption at the particle attached DNA monolayer system in aqueous solution combined with SPR imaging measurement. In the presence of colloidal Au, the SPR signal was clearly enhanced compared to that found in non-attached probe DNA monolayer.

Key words: DNA, colloidal Au, hybridization, SPR

1. INTRODUCTION

In the past decade, surface plasmon resonance (SPR) spectroscopy has been used for a simple detection as well as in-situ monitoring of DNA hybridization adsorption. [1-3] Since the SPR angle (angle of incidence at minimum reflectance) shift is induced by adsorption of molecules at a thin metallic film, the SPR method allows detection of hybridization adsorption of unlabeled target DNA in situ. [3-6] However, the SPR angle shift for the adsorption of DNA molecules is small and the sensitivity of this method is low. For the purpose of increasing its accuracy and detection limits, some efforts have been made to enhance the SPR angle shift. [3,4] However, most these method require the modification of the target molecules, so that the advantage of SPR spectroscopy is lost.

Our previous work demonstrated that the Au nanoparticle attached probe DNA monolayer could enhance the SPR angle shift (about a 3.5 times larger compared with non-attached probe DNA) without any modification of target DNA under an atmospheric condition.[8] The Au nanoparticles are attached at the top end of the probe DNA monolayer. The SPR angle is very sensitive to the surface morphology of metal particles, for example aggregation, surface defects, and distance between particles and metal surfaces. The differences of physical properties between ss-DNA monolayers, the chain length and elasticity, will affect the surface morphology of Au particles attached to probe DNA molecules. In this work, we examined the SPR response of DNA hybridization adsorption at the particle attached DNA monolayer system in aqueous solution combined with SPR imaging measurment.

2. EXPERIMENTAL

2. 1 Materials

The DNA was purchased from Nissinbo. The ss-probe DNA (BD, 30mer, 5'biotin GCAGCTTATCGT GCAATTTTAAAAGATCTT 3'Thiol), was used to form the ss-DNA monolayer on the Au thin film. The complementary ssDNA (T1, 30mer, 5' AAG ATCTTTTAAAATTGCACGATAAGCTGC 3') was used for the target DNA molecules. Au particles (diameter = 10nm) coated by straptavidin were purchased from BBI. KH_2PO_4 and K_2HPO_4 were purchased from Kanto Chemical. NaCl was obtained from Aldrich, and other reagents were purchased from Dojindo. The water used in all of the experiments was Milli-Q water.

2.2 Preparation of DNA monolayers

For SPR measurements, high refractive index glass substrate (SF10, refractive index n = 1.723) was used as sample substrates. The glass substrates were cleaned by immersing in warm piranha solution (70% concentrated sulfuric acid, 30% peroxide solution (30%)) for 1hour and rinsed with water, then blown in a nitrogen stream. The Au thin films were prepared by

sputter deposition of ca. 1 nm of Cr adhesion layer followed by ca. 50 nm of gold onto glass substrates at room temperature. The thickness of Au films was measured by the atomic force microscopy (AFM, JEOL JSPM-4210). After deposition, the film was annealed in the sputtering chamber at 200℃ for 2 min under a high vacuum condition (ca. 2.0×10^{-5} Pa). The surface of the Au thin film was cleaned by immersing in concentrated sulfuric acid solution.

DNA monolayers were prepared according to the following procedures. 4 μM (M = mol dm^{-3}) of ss-probe DNA solution (0.5 M KH_2PO_4 and K_2HPO_4, pH 7.0) was dropped onto the Au thin film and stored at 37℃ for 14 h under humid atmosphere. After rinsing with the Tris buffer solution (10 mM NaCl and 5 mM Tris-HCl, pH 7.4), the colloidal Au solution (1 M NaCl and 5 mM Tris-HCl, pH 7.4, concentration of Au particles = 4.1×10^{12} particles / ml) was dropped onto the ss-probe DNA monolayer. After rinsing with the Tris buffer, hybridization reaction was performed in 1.0 M NaCl with 10 mM Tris buffer, pH 7.4, and 1 mM EDTA. Finally, the sample substrates were rinsed with the Tris buffer. For SPR measurements, the sample substrate was set in a cell filled with the Tris buffer.

2.3 Measurements

The SPR reflectivity curve measurements were performed using a linearly polarized He-Ne laser (632.8 nm). The polarization of the laser beam was set parallel to the plane of incidence (p-polarized) by a combination of a $\lambda/2$ wave plate and Glann-Thompson polarizer. The polarized laser beam was focused onto the sample substrate through a 90° SF10 prism, contacting the sample substrate through a index matching fluid. The reflected light was detected by a photodiode and the signal was processed by a lock-in amplifier.

The scheme of the SPR imaging apparatus is shown in Fig. 1. The He-Ne laser was used as light source. The beam diameter was adjusted by a combination of 10 × objective lens (Nikon, Plan Fluor), 100 μm pinhole and a lens. The polarization of the light was set with a Glann-Thomson polarizer and a Fresnel Rhomb prism (SIGMA). The expanded laser beam was irradiated at sample substrate through the 90° SF10 prism. The reflected light from the sample substrate was captured by a charge coupled device (CCD) camera (Sony, Model DXC-151A).

3. RESULTS AND DUSCUSSION

3.1 Characterization of the probe DNA monolayer

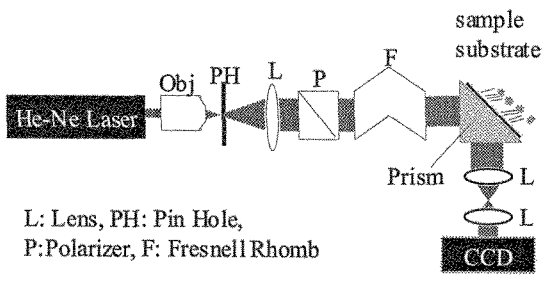

Fig.1 Schematic for SPR imaging experiment

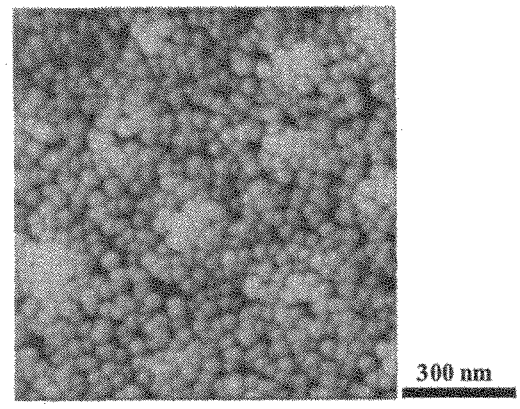

Fig. 2 AFM image of colloidal Au attached probe DNA monolayer

The Au nano-particle attached probe DNA monolayer was observed by AFM (Fig. 2). From AFM images, we could not observe particle multi-layer and the height of particles was estimated about 10 nm. This result indicated that the BD monolayer was modified with the Au particles by biotin-avidine interaction. In order to confirm the surface density of Au particles, images were obtained three positions apart from each other by 1 to 2 mm for each monolayer. Counting the particles indicated about 800 particles in the 1 μm^2 region. In contrast, we had estimated the surface density of the BD monolayer as being 14 nm^2 / molecule in our previous study.[7] Thus, almost BD molecules immobilized on the Au thin film were not attached to Au particles, and the BD monolayers can be regarded as spacer layer between the Au particles and the film surface.

3.2 SPR responses

SPR reflectivity curves of DNA monolayers are shown in Fig. 3. In the absence of Au particles, the SPR angle shift before and after the hybridization with complementary target (T1) was 0.06° . When the probe DNA monolayer was modified by Au particles, the SPR angle shift before and after the hybridization was 0.18° , and this value is about 3 times lager than that found in non-modified monolayer. Our previous work demonstrated that the Au particle attached probe DNA monolayer could enhance the SPR angle shift for the detection of DNA hybridization under an atmospheric condition.[7] Therefore, it is considered that the larger SPR angle shift observed in Fig.3 was also induced by the duplex formation of the probe DNA molecules, and the particle attached probe DNA can enhance the SPR angle shift. Thus, this method is suitable for the detection of the DNA hybridization.

As reported in previous papers, a large SPR shift is induced by a metallic nano-particle in the vicinity of metallic thin films. The angle shift depends on a refractive index surrounding the particles, and a nano-meter order separation between the particles and the film surface.[8-10] In our experimental conditions, the DNA monolayers can be regarded as spacer layer between the Au particle and the Au surface. The refractive index of ss-DNA monolayer changes by the duplex formation, because the ds-DNA molecule has a double amount of nucleotides than the ss-DNA.[11] It is also expected that

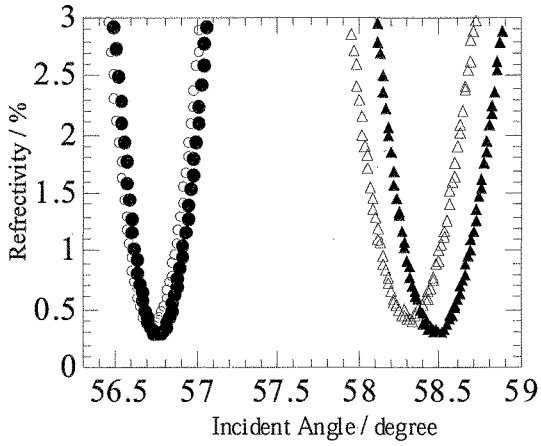

Fig. 3 SPR reflectivity curves for DNA monolayers on Au film. Open circles are for BD monolayer, filled circles are for BD-T1, open triangles are for colloidal Au attached BD, and filled triangles are for colloidal Au attached BD-T1.

average thickness would be different between the ss-DNA and ds-DNA monolayers, because the chain length of a 30 mer ss-DNA molecles is about 20.4 nm, and that of a ds-DNA is 10.2 nm. Therefore, we consider that characteristic changes (the refractive index and the layer thickness) of the ss-probe DNA monolayer, which are caused by the hybridization with the target DNA molecules, would be responsible for the larger SPR angle shift in the colloidal Au attached probe DNA monolayer.

Fig. 4 shows the SPR image of the DNA monolayers. The intensity of refraction light from the non-modified DNA region showed no intesity difference before and after the hybridization reaction. (Fig. 4 (a), (b)) In contrast, the Au particle attached probe DNA monolayer showed clear difference before and after the hybridization reaction (Fig. 4 (c), (d)). Thus, the method of Au particle modification to the probe DNA monolayer could enhance the SPR signal for DNA hybridization in aqueous solution.

4. CONCLUSION

This work demonstrates the colloidal Au attached DNA monolayer could be used for an enhance of DNA hybridization detection by SPR imaging measurement under the aquatic condition. This method possesses some potential advantages for DNA hybridization. The modification for the target DNA is not necessary. The Au particle is not phot-bleached by probe light irradiation. Hence, this method can be applied to in-situ monitoring under the enhancement of the SPR response.

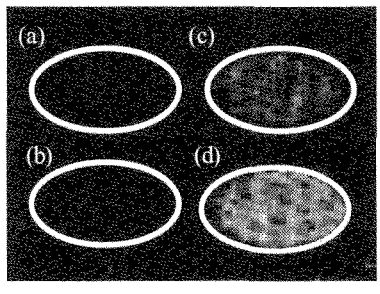

Fig. 4 SPR images of DNA monolayers. Incident angle was set the SPR angle of bare Au thin film. (a) BD monolayer. (b) BD-T1 monolayer. (c) Colloidal Au attached BD monolayer. (d) Colloidal Au attached BD-T1 monolayer.

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