Enhancement of Surface Plasmon Resonance Sensing for DNA Hybridization Using Colloidal Au Attached Probe DNA

Akira Yamaguchi,[†] Saulius Juodkazis, Shigeki Matsuo, and Hiroaki Misawa[*]
[†]*Satellite Venture Business Laboratory of Photonic Nano-Materials, The University of Tokushima, Tokushima 770-8506*
Department of Ecosystem Engineering, Graduate School of Engineering, The University of Tokushima, Tokushima 770-8506

(Received October 4, 2001; CL-010976)

In this study, we demonstrate that the Au particle modified probe DNA monolayer can enhance the surface plasmon resonance (SPR) signal for measuring hybridization of unlabeled DNA molecules. The Au particles adsorbed on single stranded (ss)- and double stranded (ds)-DNA monolayers have different optical interaction with surface of Au thin film, and this difference induces the enhancement of the SPR signal.

SPR spectroscopy is a simple optical method for observing hybridization adsorption of unlabeled DNA molecules at metal interfaces.[1] For the purpose of increasing its accuracy and detection limits, some efforts have been made to enhance the SPR signal.[2] However, most these methods require the modification of the target molecules, and the advantage of SPR spectroscopy is lost. This work aimed at developing a new technique, which would yield a large SPR angle shift (angle of incident at minimum reflectivity), and an enhanced SPR signal without the need to modify the target DNA. We propose this by a probe DNA molecule, which is modified by a colloidal Au particle at its top end, fixed on an Au thin film. In this geometry, the particle seems to be anchored by the DNA molecule, or the DNA monolayer can be regarded as a spacer layer between the particle and the film surface. It is well-known that 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.[3] The differences of physical properties between ss- and ds-DNA molecules, the chain length and elasticity,[4] will affect the surface morphology of Au particles attached to probe DNA molecules. Thus, we expect that modification of the probe DNA monolayer by Au particles would enhance the SPR angle shift caused by the hybridization. In the present study, we demonstrate that enhancement of the SPR signal can be achieved by modifying the Au particles on the ss-probe DNA monolayer. First, we constructed ss- and ds-DNA monolayers modified by Au particles, in order to confirm that the colloidal particle can enhance the SPR angle shift between the ss- and ds-DNA monolayers. Next, a probe ss-DNA monolayer modified with Au particles was prepared and the SPR angle before/after the hybridization was examined.

We used 5′- biotinylated DNA (BD) as a ss-probe DNA for the attachment of the streptavidine-albumin coated Au particle. The sequence was CACGACGTTGTAAAACGACGGCCAGA-TCAT, and the 3′- end was thiolated in order to make the self-assembled monolayer on the Au film.[5] For the hybridization reaction, complementary (T1) and non-complementary (T2) targets, composed from adenine only, were used. All DNA were purchased from Nisshinbo. DNA monolayers were prepared according to a method reported by Peterlinz with some modifications.[5] The ss-probe DNA monolayer was formed on Au thin film (50 nm thickness) by exposure to the droplet of 1 μM (M = mol dm^{-3}) probe DNA phosphate buffer solution (0.5 M KH_2PO_4 and 0.5 M K_2HPO_4, pH 7.0) for 14 h. After rinsing with R-BFR (10 mM NaCl and 5 mM Tris-HCl, pH 7.4), and drying under a moderate stream of nitrogen, hybridization was performed at room temperature in a 10 μM target DNA solution for 16 h. The hybridization reaction was performed in 1.0 M NaCl with 10 mM Tris buffer, pH 7.4, and 1 mM EDTA. After rinsing and drying under a moderate stream of nitrogen, modification of Au particles to ss- and ds-DNA monolayers was performed by exposure to streptavidine-albumin coated colloidal Au (diameter = 9.0 nm$\pm$0.7, SIGMA) in R-BFR solution for 3 h. Finally, the sample substrates were rinsed with R-BFR and dried, and immediately used in the SPR and atomic force microscopy (AFM) measurements. For the SPR measurements, p-polarized He-Ne laser light was focused onto the sample substrate through a 45° fused quartz prism, contacting the sample through glycerol. AFM measurements were performed using a SEIKO SPA 300 microscope in the non-contact mode.

Figure 1 shows the SPR reflectivity curves of the DNA monolayers. In the absence of Au particles, the SPR angle shifts from bare Au were 0.15 $\pm$ 0.01° in BD monolayer and 0.27$\pm$ 0.02° in BD/T1 monolayer (hybridization with T1). These angle shifts indicate that the most ss-BD molecules were hybridized with T1 and that the BD/T1 monolayer can be regarded as a ds-DNA monolayer, because the SPR angle shift is related to the surface amount of adsorbed DNA molecules.[1] When the Au particle was modified in respect to such ss-DNA and ds-DNA

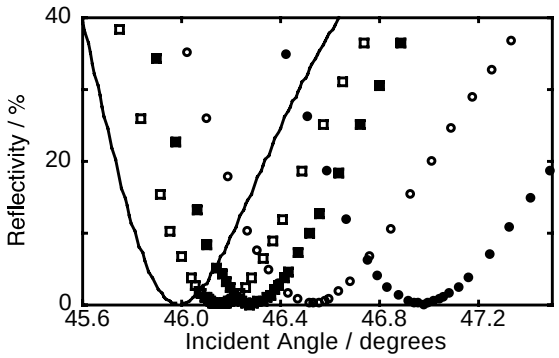

Figure 1. SPR curves for DNA monolayers on Au films. Solid line is the data for bare Au surface, open squares are for BD monolayer, and filled squares are for BD-T1, open circles are for BD/G, filled circles are for BD-T1/G, G represents the modification of Au particles. The SPR angle shifts shown in the text are average of at least three measurements.

monolayers, larger SPR angles were observed in both cases. These larger SPR angles are ascribed to the optical coupling between the particles and the film surface, as reported previously.[2b] The ds-DNA monolayer shows a larger SPR angle shift due to the attachment of Au particles (0.73°), compared to ss-DNA (0.39°). As a result, the difference of the SPR angle between the ss- and the ds-DNA monolayer with Au particles was $0.47 \pm 0.05°$, four times larger than the 0.12° found in non-modified monolayers. One should note that this shift was not observed in the case of the hybridization reaction with a non-complementary target (T2), which is not expected to form duplex with the ss-BD molecule. Therefore, it can be suggested that the Au particles adsorbed to ss- and ds-DNA monolayers have different optical interactions with the film surface.

Figure 2 depicts the AFM images of the colloidal Au particles adsorbed on the ss-and ds-DNA monolayers. We could not observe particle multi-layers in the AFM images by narrowing the scan range. Number of the particles counted in the AFM images were about 15 in the 10000 nm^2 region, and particle density was almost the same in both monolayer systems. Taking into account the area occupied by single adsorbed DNA molecule, $3.7 \times 3.7\ nm^2$,[6] it appears that most biotinylated DNA molecules were not attached to the Au particles, and that DNA monolayers can be regarded as a spacer layer between the Au particles and the film surface.

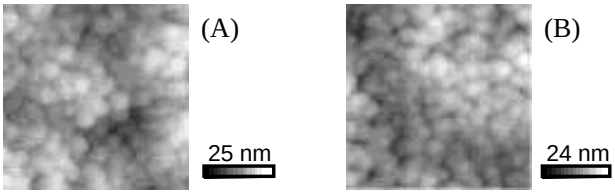

Figure 2. AFM images of Au particles attached to (A) ss-DNA and (B) ds-DNA monolayers. The image area is $0.25\ \mu m^2$.

On the basis of these experimental results, we conclude that the characteristic difference of DNA monolayers induces the change of optical interaction between the Au particles and the film surface. This change enhances the SPR angle shift between the ss- and ds-DNA monolayers. We can regard refractive index and layer thickness as the characteristic differences of DNA monolayers, because a refractive index of a medium surrounding the particles and a nm-order distance between particles and film surface can induce large SPR angle shift.[3] The refractive index in a vicinity of a gold film surface changes with the adsorption of DNA molecules, and the refractive index of the monolayer is related to the surface density of the DNA molecule.[7] Thus, the ss- and ds-DNA monolayers have different refractive indices, and this difference would be related to the SPR response of the particle modified DNA monolayers.

An average thickness would be different between ss- and ds-DNA monolayers, because the chain length of a 30-base ss-DNA molecule is about 20.4 nm and that of ds-DNA is 10.2 nm.[8] Off course, the average layer thickness is related to not only the chain length but also the conformation of DNA molecules. The DNA chains would be bent or lying on the film surface in an atmospheric condition. It is difficult to determine the exact layer thickness, but it would be related to the chain length and conformation of DNA molecule; the ss- and ds-DNA monolayers would have different average thickness. The difference of layer thickness would affect the distance between Au particles and the film surface, and this distance effect is also related to the larger SPR angle shift between ss- and ds-DNA monolayers modified with Au particles. We can not confirm the exact mechanism of the SPR response of the Au particle attached DNA monolayers, but our present work indicates that the characteristic differences of DNA monolayers are responsible for the large SPR angle shift induced by Au particle attachment.

Finally, DNA hybridization was performed using Au particle modified probe DNA monolayer. Following the formation of a ss-BD monolayer, the modification by Au particles was performed, and finally hybridization was carried out. The shape of the SPR curves was found to be almost the same as that shown in Figure 1. The SPR angle shift before/after the hybridization with a complementary target (T1) was $0.42 \pm 0.03°$, and this value was larger than that found in the non-modified DNA monolayers. In summary, we can successfully improve the SPR angle shift without modification of the target DNA molecule, and without the need for a modification process following the hybridization reaction. In this study, the SPR response was measured in an atmospheric condition, but in the future this technique can be applied for in situ hybridization detection. The modification of probe DNA using colloidal particles could provide an effective method for enhancement of SPR signal for DNA hybridization detection, and promises easy and reliable analysis.

This work was supported in part by the NEDO regional consortium project ''Development of Newly Micro-Array for Gene Analysis'', a Grant for the Collaboration between University and Society (No. 11793004) and a Grant-in Aid for Scientific Research A(2) (No. 1330518), the Foundation Advanced Technology Institute, and the Satellite Venture Business Laboratory of the University of Tokushima.

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6 By the analysis of SPR angle shift using four phase Fresnel calculation,[2a] the estimated thickness of BD/T1 monolayer was 2.3 ± 0.2 nm. From this value, surface density of 7.2×10^{12} molecules/cm^2 was calculated by assuming that the ds-DNA molecule has a rod-like structure of 10.2 nm in length, and 2 nm in diameter.[8]
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