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Study of the Interaction Between HSA and Oligo-DNA Using Total Internal Reflection Ellipsometry

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Techniques of quantitative analysis are very important for studies of the interactions between bio-molecules in the field of biotechnology and drug development. The total internal reflection ellipsometry system (TIRE) is an attractive label-free procedure for the quantitative analysis of bio-molecules because it combines the analytic ability of ellipsometry and the high surface sensitivity of surface plasmon resonance. In this work, we have used TIRE to study the optical properties of an aquatic monolayer of human serum albumin (HSA) and oligo-DNA. Also, we have monitored the adsorption and the interaction processes of protein layers.

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I. INTRODUCTION

To investigate the reaction among bio-molecules, a fluorescent label method, such as fluorescence microscopy [1], or an optical label-free method, is usually used. The typical label-free methods are surface plasmon resonance (SPR) [2,3], reflectometry interference spectroscopy [4,5], and spectroscopic ellipsometry (SE) [6]. Recently, industrial and academic applications using these optical label-free techniques have continued to grow because they can be used to analyze samples with a fluorescent substance in real time without a labeling step.

SPR and a quartz crystal microbalance [7] can monitor the adsorption and the interaction of target bio-molecules sensitively whereas they lack the capability to analyze the physical properties of the target. As an example, SPR measures only the reflectivity of p-polarized light. In contrast, SE uses two experimental quantities (ψ, Δ), which represent the reflectivity and the phase difference of polarized light [8]. Therefore, SE can be useful for analyzing both the optical properties and the structures of samples. However, biological applications of SE are limited because of its complex optical modeling and relatively low sensitivity in this context. As a way to overcome the weaknesses of this technique, total internal reflection ellipsometry (TIRE) has been proposed [9]. TIRE combines the analytic ability of SE with the high surface sensitivity of SPR. Also, TIRE can be used under an opaque ambient, such as milk, because the beam path used in the TIRE measurements does not directly pass through the opaque ambient.

Information on the interaction between Human serum albumin (HSA) and oligo-DNA and the optical properties of those materials are important inputs for pharmaceutical and medical research. HSA is commonly used for the manufacture of protein chips. However, there are only several studies of the optical properties and the physical characteristics of HSA and oligo-DNA compared with other bio molecules, such as bovine serum albumin [10–12]. Also, the majority of studies of these materials were done at a single wavelength level, and the refractive index was used merely as a necessary parameter in the measurements of the layer thicknesses. In this work, we studied the optical properties of HSA and oligo-DNA by using TIRE, and we monitored the adsorption processes of each adsorbed monolayer.

II. EXPERIMENTS

Figure 1 represents a schematic of the TIRE experimental setup and the process of adsorption of proteins. Instead of depositing a metal layer directly on the surface of the prism, we used an Au-coated glass slide to allow

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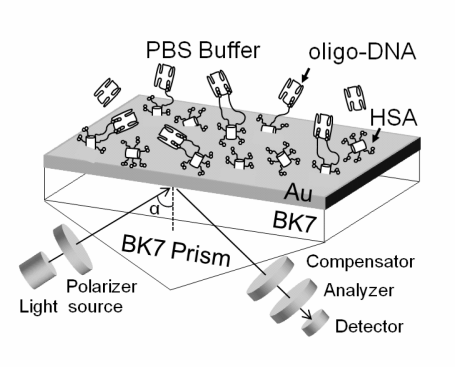

Fig. 1. Schematic of the TIRE system with a liquid transfer cell.

a convenient change of samples. An Au layer with the thickness of 50 nm is deposited on the glass slide using physical vapor deposition. The prism and the slide are made of BK7 glass, and index-matching oil ($n_d = 1.51$) is used to minimize the reflection of light from the interface between the prism and the slide glass. The TIRE liquid transfer system consists of a liquid cell with the volume of 80 μl, a degasser of 2.5 ml to remove residual gas in the liquid, and a syringe pump to control the liquid flow rate.

TIRE spectra (the ellipsometric angles ψ and Δ) were obtained in the spectral range from 400 to 1000 nm at room temperature by using a rotating-compensator SE [13] at an angle of incidence (AOI) of 75°. To remove organic residues on the Au surface, we injected 0.5M KOH into the cell for 5 min (at a rate of 500 μl/min). Phosphate buffered saline (PBS) was used as a buffer in the TIRE system. PBS was injected to rinse the inner part of the cell for 10 min (500 μl/min). Then, both the rinsing of the Au surface with PBS for 5 min (250 μl/min) and the injecting of the solution of HSA (80 μl, 10 μM in PBS) into the cell for 20 min were repeatedly carried out until the ψ and Δ values did not change. We expected these parameters not to change after the complete formation of a HSA monolayer. Next, the oligo-DNA monolayer was formed on the HSA aquatic monolayer by using a solution of oligo-DNA (80 μl, 1 mM in PBS) in the same process. Changes in the optical properties on the metal surface during the incubation step of each protein were measured by using the real-time TIRE system.

III. RESULTS AND DISCUSSION

Figures 2(a) and (b) present the TIRE spectra obtained at an AOI of 75° after the adsorption of the oligo-DNA and the multilayer model used to obtain the spectra with oligo-DNA molecules adsorbed on the HSA monolayer, respectively. In this model, the optical constants of Au and BK7 were obtained from SE measurements on a pure bulk Au sheet (99.99%) and a BK7 slide having a thickness of 1 mm. The Cauchy model are used to describe the refractive index (n), and an exponential dependence of the extinction coefficient (k) on the wavelength are assumed for each mono layer:

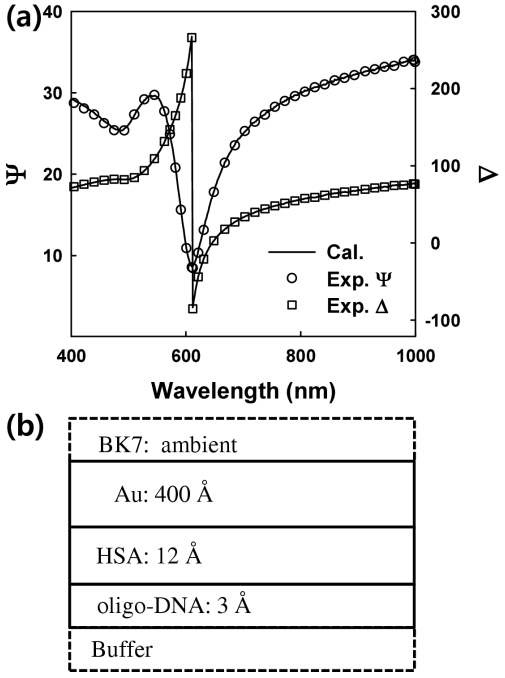

Fig. 2. (a) ψ and Δ spectra for oligo-DNA monolayer adsorbed on a HSA complex (HSA/Au/BK7 ambient) measured at a AOI of 75°. (b) Optical model.

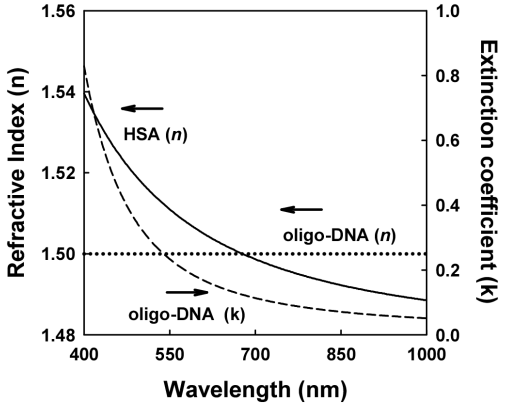

Fig. 3. Model values of refractive indices (n) of HSA and oligo-DNA, solid and dotted lines, respectively, and extinction coefficient (k) of oligo-DNA (dashed line).

$$n(\lambda) = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4},$$
$$k(\lambda) = \alpha e^{\beta/\lambda}. \quad (1)$$

Here, A, B, C, α, and β are model parameters, and λ is the vacuum wavelength of light.

The optical constants of the protein monolayer, which were obtained from the model calculation above, are shown in Fig. 3. The dashed line is the extinction co-

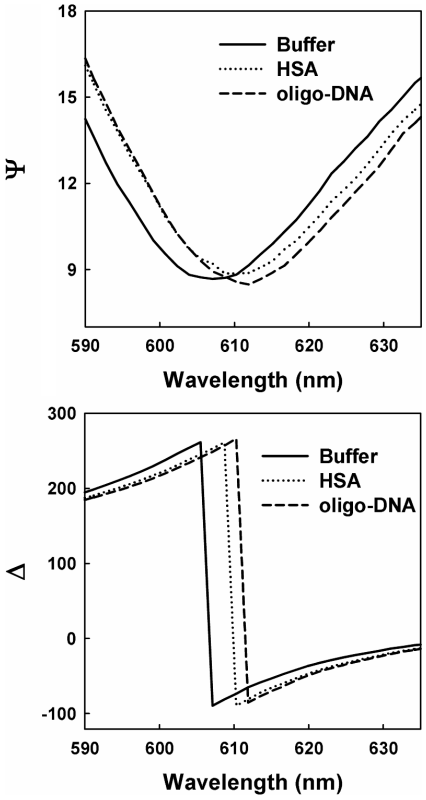

Fig. 4. Δ spectrum at AOI of 75° at the time that the HSA monolayer on a Au film (dotted) and the oligo-DNA monolayer on a HSA complex (dashed) were formed. The solid line represents the Δ spectra of the Au film under the buffer ambient.

efficient of oligo-DNA, and the solid and the dotted lines are the refractive indices of HSA and oligo-DNA, respectively. In the case of very thin transparent films, the correlation between the thickness and the optical constants is very strong. We, therefore, fixed thickness of the adsorbed oligo-DNA layer by using a model calculation with a fixed refractive index (n = 1.5). Then, only the parametrization of the extinction coefficient of the oligo-DNA layer was found from the fit of the measured spectra. The Δ parameter in the TIRE system is sensitive to structural factors, such as the thickness and the adsorption state of the protein monolayer. Figure 4 presents the Δ spectrum for an AOI of 75° when HSA (dotted) and oligo-DNA (dashed) monolayers are formed on Au film sequentially while that of the bare buffer is shown as the solid line. After each monolayer had been formed, the spectrum changed significantly, and the largest difference was observed in spectral range between 600 and 620 nm.

We also carried out real-time TIRE measurements to monitor the kinetics of the formation of protein layers at a wavelength of 604 nm. The results obtained at an AOI of 75° are shown in Fig. 5. For clarity, we show only one-fifth of the data points. The range of the highest sensitivity was found from 604 nm to 615 nm. The Δ

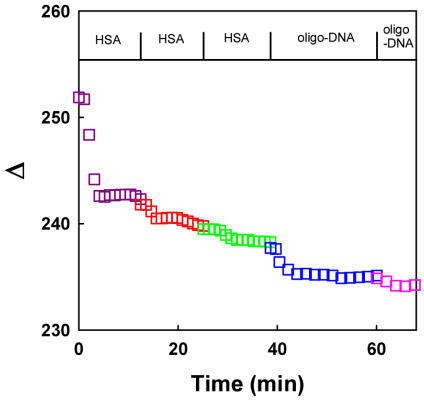

Fig. 5. (Color online) Δ values at 604 nm during the adsorption process of proteins: three injections of HSA followed by two of oligo-DNA.

parameter was remarkably changed by about 15 degree after HSA adsorption and by about 5 degree after formation of the oligo-DNA monolayer on the HSA. On the other hand, the change in ψ was several times smaller than that in Δ. As usual, the Δ parameter, which is related to the phase difference of the reflected light, is more sensitive than the ψ parameter, which is related to the amplitude of polarized light in ellipsometric measurements.

IV. CONCLUSION

We studied the optical properties of HSA and oligo-DNA proteins and monitored the adsorption between them by using TIRE. The ψ and the Δ spectra are changed remarkably as each protein monolayer is formed. We found an excellent fit to the data with an optical model and could obtain the refractive index spectrum of a protein monolayer that was not dried. Especially, the TIRE parameter (Δ) was more sensitive than other reflectivity related methods in measuring the interaction between bio-molecules. We also showed that the TIRE could be very useful for real time monitoring of the adsorption process of proteins. These results could be used in further measurements of bio-molecule interactions.

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