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Visual Sensing of Antigen-Antibody Reaction Using Polypeptide LB Films

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A polypeptide, poly(γ-benzyl L-glutamate) containing biotin group at the terminal($PBLG_{21}$-bio) was prepared. The polypeptide monolayer composed of $PBLG_{21}$-bio was deposited on a silicon substrate at surface pressure 9.5mN/m to produce its LB films. The LB films showed the structural color depending on the number of layers. The substrate was soaked into an antibody avidin solution. As the result, the maximum peak of reflection spectra of the $PBLG_{21}$-bio LB film-avidin system was shifted to higher wavelength compared with that of original $PBLG_{21}$-bio LB film. This was owing to the fact that the thickness of the LB film was increased by the adsorption of avidin. This shows a possibility of this structural color LB film for application to a visual sensing of antigen-antibody reaction.

Key words: polypeptide, LB film, structural color, antigen-antibody reaction, visual sensing

1. INTRODUCTION

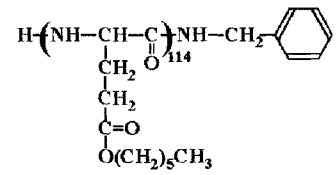
The colors of materials that we can see in our environment are generally caused by various pigments. However, in nature, insects and tropical fishes have unique color forming systems without pigments [1]. These color forming systems are originated from the interference of light. The interference of light is based on regular stratified structure whose refractive indices differ from each layers. Then the intensity of light is emphasized or enfeebled, resulting in the appearance of colors. These are called the structural color. The artificial structural color formation has been already reported on surfactant solutions and fiber systems [2-4]. However, the 2-dimensional color formation system using rod-like polymers has not been examined. We have already reported a construction of color forming LB films composed of α-helical polypeptides on the silicon substrate [5]. The structural color formation of these LB films could be characterized by the following equations [6].

$$\lambda = \frac{2h}{m}\sqrt{n^2 - \sin^2\alpha} \qquad (emphasized) \qquad (1)$$

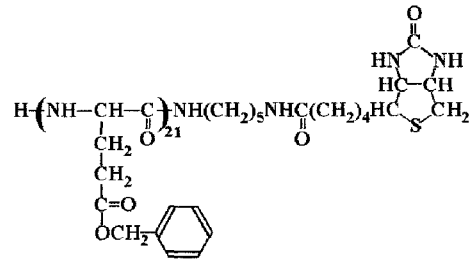
$$\lambda = \frac{4h}{2m-1}\sqrt{n^2 - \sin^2\alpha} \qquad (enfeebled) \qquad (2)$$

Where λ is wavelength of reflected light, α is incident angle, h is thickness of the layer LB film on the substrate, n is refractive index of the layer and m is natural number (m = 1, 2, ⋯). Thus, it was confirmed that the color of these LB films is dependent on the thickness and refractive index of the layer.

In this study we prepared novel polypeptides LB films containing biotin as an antigen [7, 8]. We tried to visually sense the antigen-antibody reaction using this structural color LB film.



$PHeLG_{114}$



$PBLG_{21}$-bio

Scheme 1. Chemical structures of $PHeLG_{114}$ and $PBLG_{21}$-bio.

2. EXPERIMENT

We prepared poly (n-hexyl L-glutamate) ($PHeLG_{114}$) and poly (γ-benzyl L-glutamate) containing biotin group at the terminal ($PBLG_{21}$-bio). The polymerization degree of $PHeLG_{114}$ and $PBLG_{21}$-bio was estimated by ^{1}H-NMR and gel permeation chromatography (GPC), respectively.

Silicon wafer (Nilaco) was used as substrate. The surface of silicon is known to be hydrophilic owing to the hydroxyl groups. So the silicon was modified with silane-coupling agent to produce the hydrophobic surface [9, 10]. First, substrate was cleaned by acetone and nitric acid, and then immersed in chloroform solution of octadecyltrimethoxysilane (1mM) for 24h. And then it was kept at 110 ℃ for 20 min.

The surface pressure-area (π-A) measurements of the polypeptide monolayer and the monolayer deposition were performed with LB film deposition apparatus NL-BIO40-MWCT (Nippon Laser & Electronics Lab.). $PHeLG_{114}$ (chloroform/DMF(7:3) solution) and $PBLG_{21}$-bio (chloroform solution) were spread on a Langumuir trough filled with pure water, respectively. The deposition of these monolayer was carried out at constant surface pressure, 15mN/m for $PHeLG_{114}$ and 9.5mN/m for $PBLG_{21}$-bio.

Reflection spectrum measurements were performed by use of a UV/VIS spectrophotometer V-550 (JASCO) together with an attachment, ARV-474 (JASCO).

The experiment of solvent sorption on $PHeLG_{114}$ LB film was measured in 1,4-dioxane/water solution using the UV/VIS spectrometer described above. $PBLG_{21}$-bio was soaked into aqueous solution of avidin (1.1×10^{-7}M). Antigen-antibody reaction was detected by reflection spectrum measurements. The changes in the thickness of $PBLG_{21}$-bio LB film with avidin adsorption were confirmed by an atomic force microscope(AFM) Nano-Scope IIIa(Digital Instruments).

3. RESULTS AND DISCUSSION

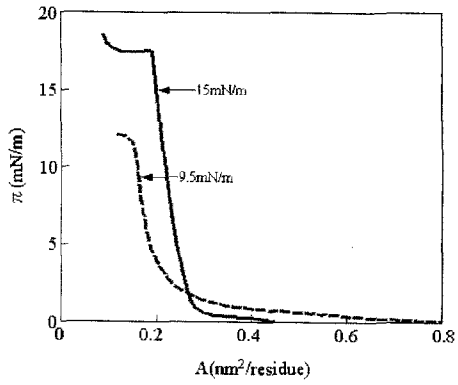


Figure 1. (π -A) isotherms for monolayers of $PHeLG_{114}$ (solid line) and $PBLG_{21}$-bio (dotted line).

Figure 1 shows the (π -A) isotherm of $PHeLG_{114}$ (a) and $PBLG_{21}$-bio (b) monolayer, respectively. Both systems formed solid state monolayer at air/water interface. The limiting area was estimated to be 0.26nm^2 / residue ($PHeLG_{114}$) and 0.21nm^2 / residue ($PBLG_{21}$-bio), respectively. This value indicated that α -helix rod of $PHeLG_{114}$ and $PBLG_{21}$-bio are lying on the air/water interface.

To create structural color substrates, we tried to transfer the several polypeptides monolayers onto the silicon substrates. $PHeLG_{114}$ was deposited at the surface pressure of 15mN/m. $PHeLG_{114}$ LB film showed structural color depending on the number of deposition, blue (80 layers), yellow (120 layers), red (160 layers). On the other hand, $PBLG_{21}$-bio monolayer was deposited at the surface pressure of 9.5mN/m. In this case, silicon substrate was annealed at 1000℃ for 3h before use to get a pre-colored substrate. 10 layers of $PBLG_{21}$-bio film on this pre-colored substrate showed viridian color. These structural color substrates change their color depending on the incident angle of light.

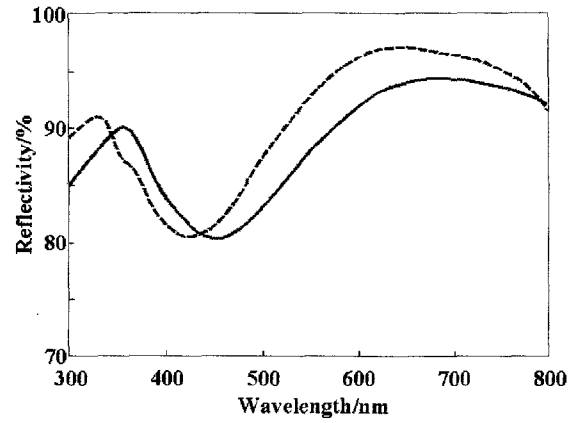


Figure 2. Reflective UV/VIS spectra of $PHeLG_{114}$ LB film (120 layers) in water (dotted line) and in 1.95M 1,4-dioxane/water solution (solid line).

These colored LB films can be characterized by their reflection spectra of LB film. Figure 2 shows reflective UV/VIS spectra of 120 layers of $PHeLG_{114}$ LB film in pure water and in 1.95M 1,4-dioxane/water solution at an incident angle of 10°. As a result of the transfer of $PHeLG_{114}$ LB film from pure water to 1,4-dioxane/water solution, maximum peak was red-shifted from 634nm to 687nm. 1,4-dioxane is good solvent for the polypeptide. It was prospective that the sorption of 1,4-dioxane caused swelling of $PHeLG_{114}$ LB film. The difference of refractive index between polypeptide (n=1.5) and 1,4-dioxane (n=1.42) is very small. So the red-shift may be owing to the solvent induced increase in the thickness

of LB film.

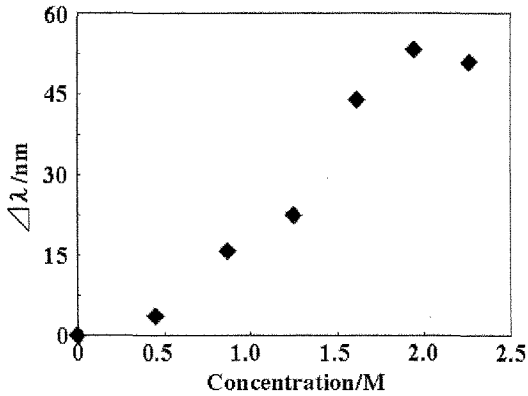


Figure 3. Changing in $\triangle\lambda$ of $PHeLG_{114}$ LB film (120 layers) with 1,4-dioxane concentration in water.

Figure 3 shows the degree of red-shift, $\triangle\lambda$, dependence on the concentration of 1,4-dioxane. The value of $\triangle\lambda$ monotonously increased with increasing the concentration of 1,4-dioxane. This implies a possibility for visual sensing of the concentration of chemicals in solution. It was also confirmed that sorption of 1,4-dioxane vapor on $PHeLG_{114}$ LB film drastically changed the structural color. It should be noted that this color change occurred homogeneously over the whole area of the LB film.

It was confirmed, therefore that the sorption of solvent causes structural color change. However, it is difficult to specifically detect a chemical among the mixture. So as a next step, we tried to construct the visual sensing system for antigen-antibody reaction.

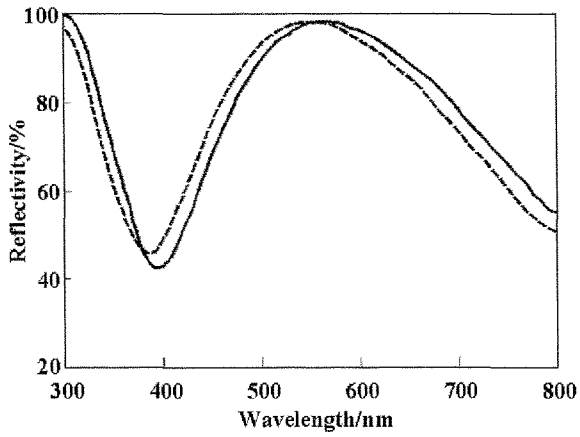


Figure 4. Reflective UV/VIS spectra of SiO_2 layer with 10 layers of $PBLG_{21}$-bio (dotted line) and after soaking avidin/water solution (solid line).

Figure 4 shows reflective UV-VIS spectra of $PBLG_{21}$-bio LB film (10 layers) on the pre-colored substrate before and after the soaking into avidin/water solution (1.1×10^{-7}M 3h) at an incident angle of 10°. It is clear that the maximum peak of the spectra was shifted 20nm longer wavelength. Eq.(1) shows that 20nm red-shift corresponds to 7.2nm increasing in the thickness of the LB film.

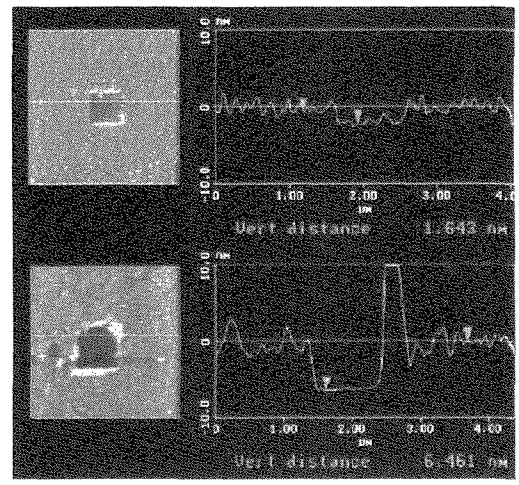


Figure 5. AFM images of 1 layer of $PBLG_{21}$-bio LB film and after soaking avidin/water solution on Si/SiO_2 surface.

AFM images (Figure 5) show the change in the thickness of $PBLG_{21}$-bio LB film before and after soaking avidin/water solution. The increasing in the thickness was estimated to be about 5nm. Considering that the diameter of avidin is about 6nm, it was expected avidin adsorbed on the biotin site on the surface of $PBLG_{21}$-bio LB film. This may be a reason for the structural color change. This result also shows the possibility of visual sensing of antigen-antibody reaction.

4. CONCLUSION

In this study, we succeeded in preparation of structural color LB films with α–helical hydrophobic polypeptide and α–helical hydrophobic polypeptide containing biotin group on the silicon substrate. They showed various interference colors depending on the number of layers. The structural color could be changed by sorption of 1,4-dioxane which caused swelling of $PHeLG_{114}$ LB film. This red-shift was dependent on the 1,4-dioxane concentration. On the other hand, the substrate prepared by using α–helical polypeptide containing biotin group, changed its structural color by adsorption of avidin

owing to the increasing in the thickness. This shows a possibility of visual sensing for antigen-antibody reaction.

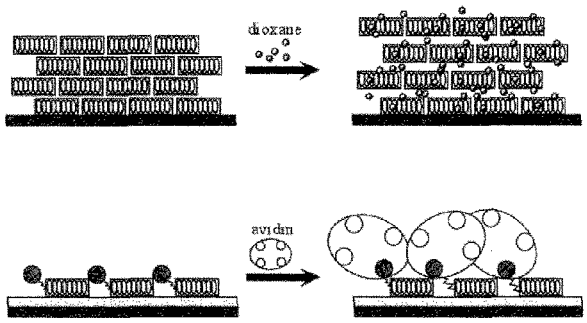


Figure 6. Systems of changing structural color caused by 1,4-dioxane sorption and avidin adsorption.

The results obtained in this study may be applicable to novel visual sensing systems for the host-guest chemistry.

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