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Development of Sensing System for Antigen-anitibody Reaction Based on Interference Color System

Masayoshi Tanaka, Ai Saito, Ryojiro Tominaga and Takatoshi Kinoshita*
Graduate School of Engineering Nagoya Institute of Technology,
Gokiso-cho, Showa-ku, Nagoya 466-8555, Japan
Fax: 81-52-735-5267, e-mail: kinoshita.takatoshi@nitech.ac.jp

We have demonstrated sensing for Bovine Serum Albumin (BSA) by interference color system. Uniform layer of anti-BSA formed on SiO_2/silicone thin-layers, which showed the interference peak in visible light region, was successfully utilized for BSA sensing by reflective spectrum. BSA was detected over 1.0×10^{-8} M by interference color system. Development of the antigen sensing system without special reagent for second antibody having fluorescent moiety would lead to construct a novel immunoassay construction.
Key words: Interference color, Sensing of biological molecule, Antigen-antibody reaction

1. INTRODUCTION

Detection of bio-related small molecules such as antigen is of great importance for immunoassays. One of the most practical tools for the detection is utilized by solid phase manner. Most of the solid phase immunoassays that have been well developed so far are required to use the second antibody labeling with fluorescent agent or enzyme to give immune information by color change.[1-5] Since the antigen-antibody reaction is highly sensitive, these sensing methods have much potential to be applied in medical and pharmaceutical fields. On one hand, necessity of the second and third reaction for pigmentation of the system is one of the biggest problems in these methods. Secondary antibody reaction and additional enzyme reaction for the pigmentation will require many steps and much time. Therefore, it is required to develop a novel method for one-step visible detection of site-specific molecular interaction without any fluorescent agents and enzymes.

Interference color is well known as non-pigments coloring system. It would be applied as a sensing system, since the color changes were proportionally related to the thickness of the constructed layer.[6-7] Previously, we have demonstrated that a silica thin-film constructed on silicon substrate showed interference color change, when bacteria and protein adsorbed on it.[8-12] Adsorption of bacteria having sub-micron scale indicated visible color change, which was able to be estimated not only by spectrum measurement, but also by the naked eyes. Adsorption of biological molecules such as antigen, proteins and peptides with nanometer scale would be the next feature for the development of visible sensing system.

In this study, we will describe about the detection of antigen as a small biological molecule by the interference color system. By the preparation of antibody layer which can selectively bind with antigen, we tried to construct sensing system for antigen adopting thickness control by selective binding of antigen with antibody.

2. EXPERIMENTAL PROCEDURE

Glycidoxy-modified silica thin-film Disc form silicon wafer (7mm diameter (100)) was annealed at 960~1080°C for 5 hours by electric furnace to make silica thin-layer. Prepared thin-layer was deterged by the immersion into 10 % ammonia / 2.7% hydrogen peroxide / Milli-Q water solution for 30 minutes and completely dried. Deterged silica thin-film was immersed into 1% of 3-glycidoxypropyl triethoxysilane (GPTES) / ethanol solution for 1 day at room temperature. After the immersion, thin-film was picked up and annealed at 110°C for 20 minutes to stabilize the Si-O bond.[13]

Anti-BSA modified substrate Avidin / PBS solution (1.5×10^{-6}M, pH 7.4) and biotin modified IgG (anti-BSA) / PBS solution (6.6×10^{-6} M, pH 7.4) are prepared. First, the glycidoxy-modified silica

thin-film was immersed into the avidin/PBS solution for 1 hour at room temperature. Configured substrate which was covalently attached with avidin was washed with pure PBS, and then it was immersed into the anti-BSA/PBS solution for 1 hour at room temperature.

Sensing investigation of BSA The anti-BSA modified substrate was immersed into BSA/PBS solution (1.0×10^{-10}~1.0×10^{-5} M, pH 7.4) for 3 hours. The substrate washed with pure PBS buffer solution was dried under atmospheric condition. For amplifying procedure, the substrate was immersed into IgG solution (6.6×10^{-6} M, pH 7.4) for 3 hours, and washed with PBS buffer prior to analyze for reflective VIS spectrum.

Reflective VIS measurement Reflective VIS spectrum measurements were performed by LIFES-5501 (MORITEX). White light incidence from optical fiber was reflected on the sample (angle of incidence: 15°), and the reflected light was analyzed with spectroscopy. The spectrum of bare Si wafer was measured as a background prior to use.

Considering that the light reflection at the protein/silica interface is negligible, it was possible to discuss equation 1 brought by Bragg's law and Snell's law. It is clear that the color condition (λ) depends on the thickness of protein (h_1) and silica (h_2), refractive index of the protein layer (n_1) and silica (n_2), and incident angle (α).

$$\lambda=4h_1(n_1^2-\sin^2\alpha)^{1/2}/m+4h_2(n_2^2-\sin^2\alpha)^{1/2}/m \quad (1)$$

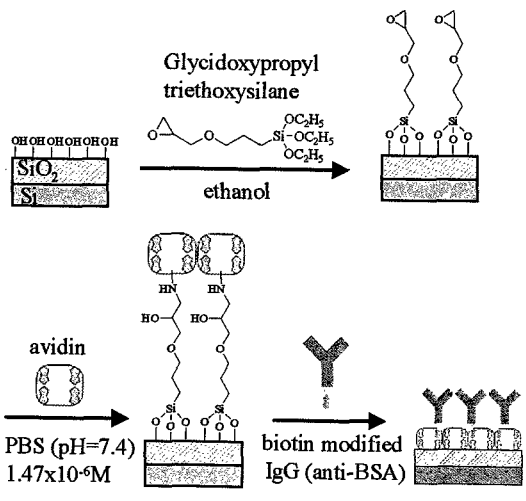

Figure1. Schematic illustration of construction of anti-BSA modified substrate.

3. RESULTS AND DISCUSSION

As a base substrate showing interference color, we have prepared a silica/silicon laminated film by thermal oxidation method (Figure 1). Annealing of silicon wafer at 960~1120°C for 5 hours brought oxidization of wafer surface and uniform silica layer was constructed. Since the thickness of the formed silica thin-layer was sub-micron order which was corresponding to the region of visible light, thickness change with adsorption of ingredients could be detect by reflective VIS spectrum. Taking advantage of the reactivity of the silica surface, the surface modification was readily achieved by treatment with silane-coupling reagents. Glycidoxyl group would react with amino group and carboxyl group of avidin to give covalently attached layer on its surface. By immersing the glycidoxy-modified substrate into avidin solution, avidin was immobilized onto the substrate which was confirmed by shift of the reflective spectrum. The obtained avidin-immobilized substrate was adopted to avoid unexpected disassembly of the adsorbed ingredients. After immersed the avidin-immobilized substrate into 6.6×10^{-6} M of biotin-modified IgG (anti-BSA) solution, definite red-shift by 8.0 nm was observed in reflective VIS spectra. Owing to the high association constant between biotin and avidin (1.0×10^{15} M^{-1}), biotin-modified IgG would regularly bind with avidin layer. This minimum peak is corresponds to the m=3 in equation 1. Refractive index of avidin and anti-BSA is expected to be similar value with SiO_2 (1.46). By the simulating of this peak change with assuming the same index layer thickness changes without other deflection, it corresponds to the 4.1 nm of thickness increasing. The obtained thickness was almost agreed with the width of the anti-BSA, suggesting that the immobilized anti-BSA was laid on the substrate after drying the substrate. The increase of the thickness no longer observed when the substrate immersed into anti-BSA solution with higher concentration. It was suggested that a steric hindrance between IgG unit was affected the binding state of the anti-BSA.

Detection of antigen was performed by means of BSA, which specifically binds with anti-BSA (Figure 2). The anti-BSA modified substrate was

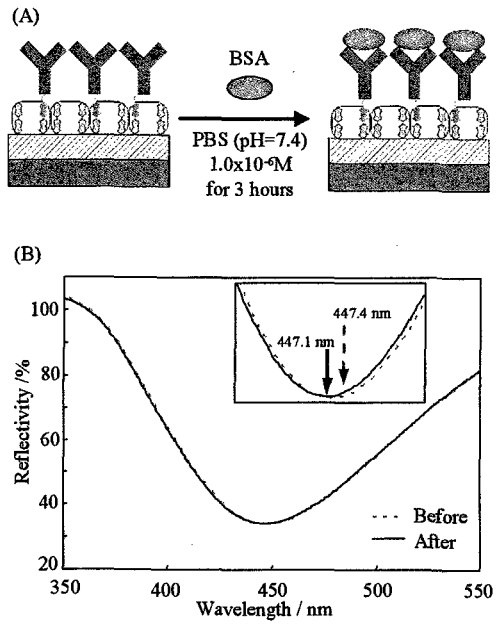

Figure2. (A)Schematic illustration of the sensing experiment (B) Reflective VIS spectra of the anti-BSA modified substrate before and after immersion into BSA solution.

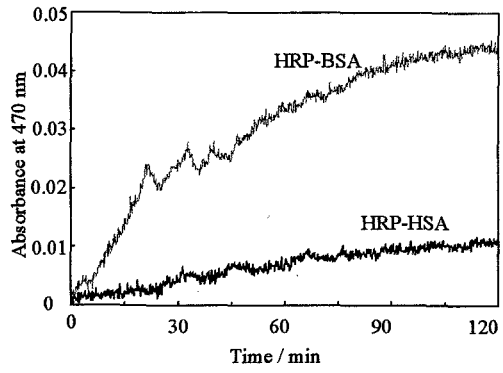

Figure3. Plots of absorbance at 470 nm for the estimation of binding on the antibody modified substrate with enzyme-labeled BSA

immersed into BSA solution. The minimum peak of the anti-BSA modified substrate observed at 447.1 nm was shifted to 447.4 nm. The shift was only 0.3 nm, which was obviously smaller than the theoretical value. In order to confirm binding of the BSA on the substrate, an enzyme-tethered BSA was used instead of BSA. After immersion of anti-BSA modified substrate into Horse radish peroxidase (HRP)-tethered BSA /PBS solution (pH 7.4) for 3 hours, substrate was carefully washed. Then, the obtained substrate was immersed into the solution which induced enzyme reaction of HRP. When guaiacol coexisted with H_2O_2 and HRP, it transformed into tetra-guaiacol to give an increase of absorbance at 470 nm in UV-vis spectrum. The plot of the absorbance at 470 nm was shown in Figure 3. The absorbance was gradually increased in the solution immersing with HRP-BSA treated substrate. On the other hand, the substrate immersed into HRP-tethered Human Serum Albumin (HSA) solution little indicated increase of absorbance at 470 nm. These results were suggested that BSA was specifically bound with anti-BSA on the substrate. Thus, the observed little shift of the minimum peak would be attributed that BSA was bound with anti-BSA, but it did not affect to thickness increase. Since the width of anti-BSA, which laid on the substrate after drying, was almost same as size of BSA, the thickness change could not be expected even when BSA was specifically bound with anti-BSA.

In order to amplify the shift of the minimum peak, anti-BSA was layered up onto the BSA layer as shown in Figure 4. The minimum peak of the anti-BSA modified substrate observed at 447.1

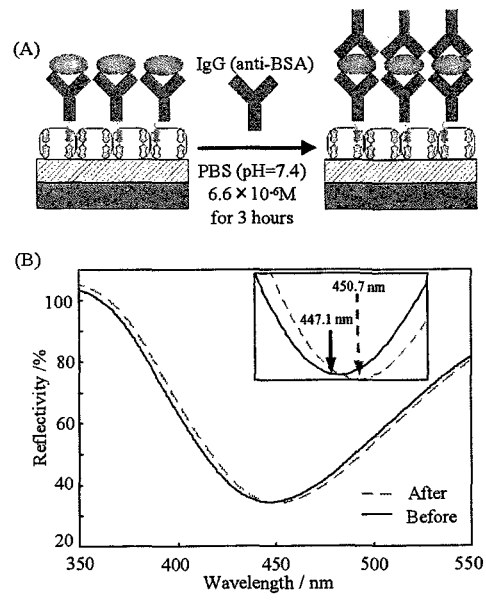

Figure4. (A) Schematic illustration of the sensing experiment. (B)Reflective VIS spectra of the anti-BSA modified substrate before and after immersion into BSA solution.

nm was shifted to 450.7 nm, indicating obvious increase of the shift range. The observed shift was corresponded to thickness increase by 1.9 nm. However, it was smaller than the width of the IgG, suggesting that IgG-BSA layers on the substrate was partially increased by second reaction with IgG to give the change in interference of the visible light. BSA binding was detected by 1.0 x 10^{-8} M as shown in Figure 5. The detection of antigen was successfully carried out by ELISA like method with non-pigment amplifier.

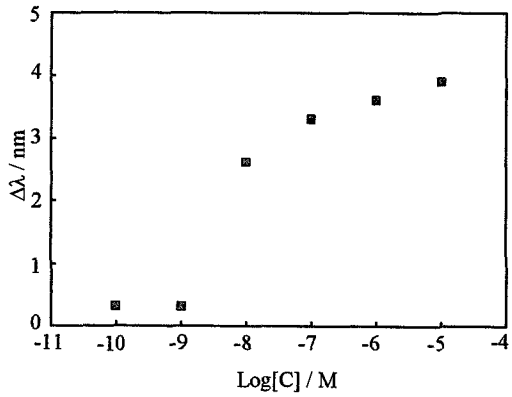

Figure5. Plot for Δλ of the minimum peak and concentration of BSA solution

4. CONCLUSION

In this study, we have demonstrated sensing for antigen by interference color system. Uniform antibody layer formed on SiO_2 thin-layers showed the interference in visible light region. The obtained substrate successfully utilized for antigen sensing by reflective spectrum, though second reaction with standard antibody required. The color change observed in the combination with BSA and anti-BSA was occurred over 1.0×10^{-8} M of BSA concentration. Such non-pigment sensing system would reduce the cost to sense the allergy, diseases, and virus, compared with the method requires second antibody labeling with fluorescent reagent or enzyme, such as ELISA or Western blotting. These optical properties of self-assembling layered system might be a hint for novel immunoassay construction.

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