Macromolecular Research, Vol. 14, No. 4, pp 483-485 (2006)

Micro-patterned Polydiacetylene Vesicle Chips for Detecting Protein-Protein Interactions

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Received April 24, 2006; Revised June 7, 2006

Introduction

Advancement of conjugated polydiacetylene (PDA) vesicles as chemical and biological sensors has attracted great interests due to their unique chromatic properties. Monomeric diacetylene lipids, such as 10, 12-pentacosadiynoic acid (PCDA), can undergo polymerization via 1,4-addition reaction upon UV light to form ene-yne alternating polymer chains, producing liposome-like vesicles. The vesicles show a bichromic property from blue to red upon external perturbations, such as heat, pH, mechanical stress, and solvents.[1-4] Employing their unique property, PDA's have been developed as specific and convenient biosensors. For example, PDA-based sensors have successfully detected influenza virus, Cholera toxin, *Escherichia coli*, oligonucleotides, lipopolysaccharides, antibodies, and antigens.[5-13] However, most of the applications of PDA as biosensors have been carried out in aqueous solution, which requires large amount of vesicles, antibodies, and analytes. Due to these limitations, micro-patterned biosensors immobilized on a solid-state material, called as biochips, have gained much attention. Recently, we have demonstrated micro-arrayed PDA systems could be utilized as fluorescence-based sensor chips for external stimulations.[3,14,15] Here, we developed a prototype of a protein chip using micro-patterned PDA vesicles, which can detect protein-protein interactions.

Experimental

Materials. 10,12-Pentacosadiynoic acid (PCDA) was purchased from GFS chemicals. PCDA-Biotin was prepared as described in the literature.[16] Synthesis of PCDA-EDEA-SA-NHS will be reported elsewhere. Polyclonal primary antibody produced by a rabbit for detecting an *E. coli* surface protein was purchased from Fitzgerald Industries International, Inc. The fluorescein isothiocyanate (FITC)-conjugated anti-rabbit and anti-mouse secondary antibodies were purchased from Sigma Aldrich Co.

Preparation of Lipid Vesicles. The diacetylene monomers were dissolved in chloroform and the solvent was removed by purging with N_2 to generate a thin lipid film on the glass surface. A buffer solution (HEPES, 5 mM, pH=8.0) was added to yield a total PCDA lipid concentration of 1.0 mM. The samples were then heated at 80 °C for 15 min and sonicated (Fisher Sonic Dismembrator Model 550 W, 25% of the power) for 15 min. The resulting solution was filtered through a 0.8 μm PTFE filter and the filtrate was cooled at 4 °C for 12 h.

Preparation of Avidin-Coated Glass Slides. A proper quantity of Biotin-NHS was added to mixture of PBS buffer and DMSO. Amine-coated glass slides were reacted with Biotin-NHS solution for at least 4 h at room temperature. Then the glass slides were immersed into avidin solution, which contains avidin in PBS buffer, and reacted for 1 h at room temperature to make avidin-coated glass slides.

Preparation and Immobilization of Antibody-conjugated PDA Vesicles on Glass Slides. To conjugate the primary antibody to the PDA vesicles, 6 μL primary antibody solution with 1.0 mg/mL concentration was added into 200 μL liposome solution. The solution was incubated at room temperature for 4 h. The prepared vesicle solution was spotted onto avidin-coated glass slides using Nano-plotter v 1.2 (GeSim, German) and then was immobilized at 37 °C for 2 h. After immobilization, the glass slides were washed in deionized water for 1 min and the vesicles were polymerized by the exposure to 254 nm UV light at the intensity of 1 mW/cm^2 for 3 min.

Detection of Interactions between Primary and Secondary Antibodies. To detect a protein-protein interaction, 7 μL FITC-conjugated anti-rabbit secondary antibody solution with 150 μg/mL concentration was dispersed on the glass slides, covered by a cover glass and incubated at room temperature for 4 h. The slides were washed in deionized water three times. The fluorescence levels of the vesicles were observed with Olympus BX51.

Results and Discussion

Three diacetylene lipids, 10, 12-pentacosadiynoic acid (PCDA), PCDA-EDEA-SA-NHS and PCDA-Biotin, were dissolved in 1 mL chloroform by 8.5 : 1 : 0.5 molar ratio (Fig-

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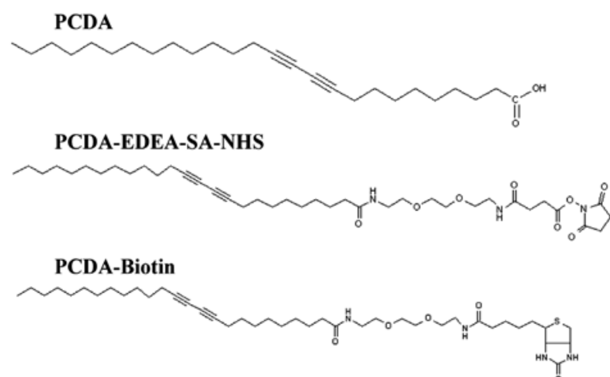

Figure 1. Molecular structures of the lipid monomers used in the experiment. From the top, the structures of 10,12-pentacosadiynoic acid (PCDA), PCDA-EDEA-SA-NHS, PCDA-Biotin are illustrated.

ure 1). The PCDA mixtures were processed as previously described to make vesicles and reacted with primary antibodies. The solution containing the vesicles was spotted onto avidin-coated glass slides and immobilized by biotin-avidin binding. The vesicles were polymerized by the exposure to UV light. Then, the anti-rabbit secondary antibody solution was dispersed and hybridized on the glass slides (Figure 2).

The fluorescence levels of the vesicles and the secondary antibodies were monitored using filters of the Olympus microscope. Because the secondary antibodies were labeled with fluorescein, they were monitored on a green filter at 515 nm (Figure 3(B)). It was reported that when PDA vesicles were perturbed, the emission light of the fluorescent vesicles was detected at 580 nm.[15] After the secondary antibodies were reacted with primary antibodies on the vesicles, the vesicles turned to be fluorescent on a red filter (Figure 3(C)). The images obtained on two filters were overlapped, suggesting that the vesicles changed their fluorescence property by binding with secondary antibodies. Through heating of the glass slide that gives a strong perturbation to the vesicles, the presence of PDA vesicles was confirmed (Figure 3(D)). The experiment was repeated three times and similar results were obtained each time.

To prove that the binding between PDA vesicles and secondary antibodies was mediated by primary antibodies, two control experiments were carried out. The first was a reaction between the vesicles without primary antibodies and FITC-labeled anti-rabbit secondary antibodies. When the vesicles without primary antibodies were reacted with the anti-rabbit secondary antibodies, no fluorescence was observed on the glass slide (Figure 4(A)), suggesting that no external perturbation was made on the vesicles. Without primary antibodies, the interactions between the vesicles and the secondary antibodies were not made. By heating the glass slide, the

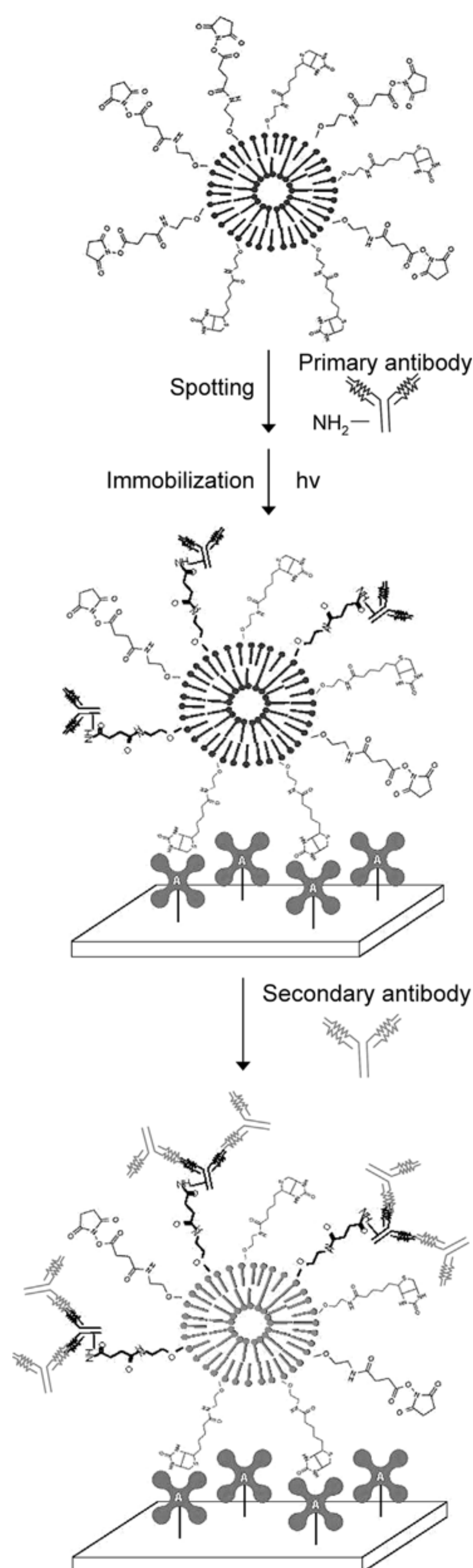

Figure 2. Strategy for preparing PDA vesicles immobilized on the avidin-coated glass slide and for detecting interactions between primary and secondary antibodies.

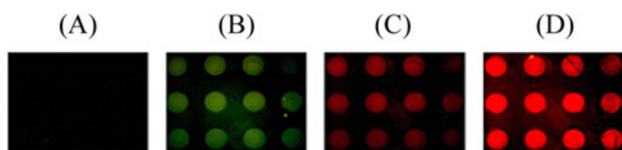

Figure 3. Fluorescent images of micro-patterned PDA vesicles (A) before the experiment, (B) after incubated with FITC-labeled anti-rabbit secondary antibodies monitored on a green filter (515 nm), (C) on a red filter (580 nm), and (D) after 5 min heating. (A) and (D) images were taken on a red filter, and the diameter of spots is about 200 μm.

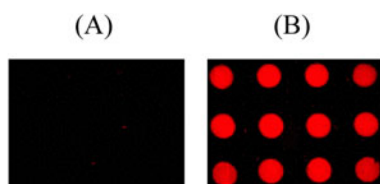

Figure 4. Fluorescent images of micro-patterned PDA vesicles without primary antibodies (A) after incubation with FITC-labeled anti-rabbit secondary antibodies on a red filter (580 nm) and (B) after 5 min heating (red filter).

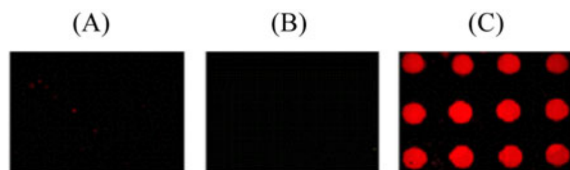

Figure 5. Fluorescent images of micro-patterned PDA vesicles (A) after incubation with FITC-labeled anti-mouse secondary antibodies on a green filter (515 nm), (B) on a red filter (580 nm), and (C) after 5 min heating (red filter).

presence of the vesicles was also confirmed (Figure 4(B)).

The other control experiment was the use of FITC-labeled anti-mouse secondary antibodies in the reaction. The secondary antibodies for mouse-origin proteins should not interact with primary antibodies produced by a rabbit. The result was in accordance to our expectation and no fluorescence was detected by the reaction on either green or red filter (Figure 5). The two control experiments proved that no fluorescence level change was made when the primary antibodies on the vesicles did not interact with secondary ones in the solution.

Conclusions

In conclusion, micro-patterned PDA vesicles were proved as a biosensor to detect a protein-protein interaction. The vesicles were conjugated with primary antibodies and immobilized on glass slides as an array form. The fluorescence level of the PDA vesicles was changed by protein-protein interactions between primary and secondary antibodies. This experiment proves that the micro-patterned PDA vesicles are useful to detect protein level interactions and can serve as a novel substrate to develop a protein chip.

Acknowledgments. This research was supported partly by the Korea Research Foundation (KRF-2004-005-D00057), the Ministry of Science and Technology (Protein Chip Technology program), and the Brain Korea 21 Project. K.-W. Kim and H. Choi equally contributed to this work.

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