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

Kyung-Woo Kim, Hyun Choi, Gil Sun Lee, Dong June Ahn*, and Min-Kyu Oh*

Department of Chemical and Biological Engineering, Korea University, Seoul 136-713, Korea

Jong-Man Kim

Department of Chemical Engineering, Hanyang University, Seoul 133-791, Korea

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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-

*Corresponding Authors. E-mail: mkoh@korea.ac.kr, ahn@korea.ac.kr