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.5-11 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.11,14,15 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 N2 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/cm² 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-