Detection of α-Cyclodextrin and *E.coli* Cell Using Polydiacetylene Supramolecules

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Introduction

The surface of a cell membrane is a mosaic of highly specific molecular recognition receptor sites. Recently, diacetylene molecules have been of great interest to mimic the self-organization and functionalization of the cell membrane. Self-organization of diacetylene monomers into supramolecular assemblies gives rise to prepare polymerized vesicles and films by irradiation with 254 nm UV light. These supramolecules initially blue undergo a visible color transition from blue to red by various environmental perturbation including temperature, pH, solvent, and mechanical stress [1-2]. The color transition occurs when external stimuli impose stresses altering delocalization length of π electrons along polydiacetylene backbones. It has been reported by Charych et al. in 1993 that by chemically connecting a special receptor to the diacetylene molecule and inserting the obtained probe into a diacetylene matrix, the color of such kind of assay will change from blue to red once it adsorbed biomolecules and chemicals. Recently we were successful in immobilization of the PDA liposomes on glass substrates without losing their unique color changing [3]. Also Polydiacetylenes (PDAs) are unique in terms of their output flourescence emission signals, which range from nothing to red in response to environmental perturbations.

Many studies using polydiacetylene supramolecules have been reported mostly about detection either in vesicle solutions or in LB films. In this study, for the first time we immobilized and patterned PDA vesicles on solid substrate using micro arrayer, which have moieties to react with chemical and biological materials. Immobilized vesicle system was developed since it possesses many advantages in multiple screening, durable stability, and higher sensitivity. We applied polydiacetylene supramolecules to chemical and biological sensors for detection of α-cyclodextrin and *E.coli* cell selectively.

Experimental

Materials. 10,12-Pentacosadiynoic acid (PCDA) was purchased from GFS chemicals. PCDA-ABA and PCDA-Biotin were synthesized prepared as described in the previous paper [4]. Polyclonal primary antibody produced by a rabbit for detecting an *E. coli* surface protein was purchased from Fitzgerald Industries International, Inc.

Preparation of PDA Vesicles. The diactyelene mixed 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 deionized water was added to yield a total PDA lipid concentration of 1.0 mM. The samples were then heated at 80°C for 15 min and sonicated (Fisher Sonic Dismembrator Model 550W, 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.

Glass Preparation. Slide glass was coated with biotin for 2 h and with avidin for another 2 h because biotin was critical to immobilize avidin, which was required to immobilize polydiacetylene supramolecules (vesicles).

A nano-plotter microarrayer from Gesim was used to pattern supramolecules on the slide glass surface which was coated with avidin/biotin prior to use of the microarrayer. The supramolecules were deposited on the avidin modified surface, which was followed by rinsing with deionized water. Then the supramolecules/avidin modified surface hybrid structure was exposed to UV light (254 nm) at an intensity of 1 mW/cm^2 for polymerization. Finally, the supramolecules were immobilized and chemisorbed on avidin modified surface.

***E.coli* Detection Experiment.** Avidin was used to bind patterned vesicles and anti-*E.coli* antibody, so patterned vesicle substrate was dippied into avidin solution for 2 h and anti-*E.coli* antibody solution for 2 h in sequence. Four cells were used in this experiment. (*Escherichia coli, Bacillus subtilis, Listeria monocytogenes, Salmonella typhimurium.)*

Results and discusstion

α-cyclodextrin Detection. CDs are three kinds of cyclodextrins such as α-CD, β-CD, and ɣ-CD which have six, seven, and eight glucose units, respectively. These CDs form host-guest inclusion complex with specific supramolecules. Figure 1 shows that self-emitting red fluorescence was induced in only the reaction between PDA vesicles and α-CD, however there was no fluorescence signal in case of β and ɣ-CDs. These results conform that only α-CD is able to form inclusion complex with PDA vesicles. By heating the glass slide, the presence of the PDA vesicles was also conformed.

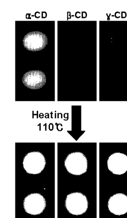

Figure 1. Fluorescent images of micro-patterned PDA vesicles (top) after reaction with α-CD, β-CD, and ɣ-CD for 60 min, and (bottom) after 5 min heating.

***E.coli* Cell Detection.** Figure 2 indicates that immobilized PDA vesicles could selectively detect only *E.coli a*mong *Escherichia coli, Bacillus subtilis, Listeria monocytogenes, Salmonella typhimurium*,, based on the fact that PDA vesicles showed self-emitted red fluorescence in reaction with *E.coli*, but no fluorescence against *Bacillus subtillus* and so on. In addition, *E.coli* without antibody moiety did not induce fluorescence signal. This means that fluorescence signal was induced only by the reaction between *E.coli* and *E.coli* primary antibodies. By heating the glass slide, the presence of the PDA vesicles was conformed. These results strongly suggest that the PDA vesicles have great potentials to be applied to a label free cell chip which can detect various pathogens.

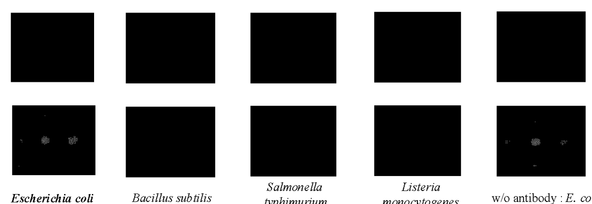

Figure 2. Fluorescent images of micro-patterned PDA vesicles with E.coli primary antibodies (top) after incubation with various cells for 5 h and (bottom) after 5 min heating, and (right column) PDA vesicles without primary antibody with E.coli cell.

Conclusions

This detection method could be applied as DNA chip, protein chip, and cell chip for multiple screening as well as chemical sensor by modifying the functional groups of diacetylene monomer.

References

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