Conjugated Diacetylene Supramolecules for Label-Free Biological Sensors and Chips

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Introduction

Polydiacetylene (PDA) supramolecules are interesting biomimetic materials in view of application to chemical and biological sensors. These conjugated supramolecules are unique in changing color from blue to red upon specific binding events, due to shortening of conjugation length of π-electrons along backbones. Various binding events including viruses, toxins, glucose, and ionic interactions have been reported detectible [1-2]. However, simultaneous screening of various binding events has not been possible with solution-phase liposomes and solid-supported films of polydiacetylenes. As a first step to endow these systems with multiple-screening function, we were successful in immobilization of the polydiacetylene liposomes on solid substrates without losing their unique color changing property [3]. It has been known that the 'blue-phase' polydiacetylenes are non-fluorescent while the 'red-phase' ones are fluorescent. It is surprising that not many works have been devoted to the development of polydiacetylene sensors based on fluorescence signals [4-5]. In this study, we focus on fabrication of polydiacetylene supramolecule dot array patterns on solid substrates by using a conventional microarrayer. Each dot is found to possess the color-changing property as well as the fluorescence self-emission. This technique allows us, for the first time, to fabricate biochips based on polydiacetylene supramolecules. Label-free detection of small molecules and biological target analytes is demonstrated.

Experimental

Materials. 10,12-pentacosadiynoic acid (PCDA) was purchased from GFS Chemicals. Other diacetylene derivative monomers were prepared according to the literature procedures.

Preparation of Diacetylene Liposome Solution. A mixture of the diacetylene monomers was dissolved in chloroform in a test tube. The solvent was evaporated by a stream of N_2 gas and HEPES (5 mM, pH 8.0) buffer was added to the test tube to give the desired concentration of lipid (1 mM). The resultant suspension was sonicated (Fisher sonic dismembrator model 550) for 15 min at a temperature of around 80 °C. Following sonication, the solution was filtered to remove dispersed lipid aggregates by using 0.8 μm filter and cooled at 4 °C for overnight.

Formation of Polydiacetylene Array Patterns. Properly modified glass substrates were placed in a conventional ink-jet microarrayer. Diacetylene liposomes functionalized with proper chemical and/or biological ligands were then transferred to the glass substrates using this standard array spotter to generate surface patterns. After rinsing the glass substrates with deionized water, the patterned glasses was irradiated with 254 nm UV light (1 mW/ cm^2) for designated periods of time to generate polymerized dot arrays which are initially nonfluorescent.

Results and discussion

Firstly, we focused on the feasibility of generation of patterned PDA images using a micro array spotter. The arrayed sensor system would be more versatile and practical in the chip-sensor system. Accordingly, liposome solutions prepared with a 1:1 mixture of the two diacetylenic lipid monomers, PCDA-EDEA and PCDA-EDA were transferred to aldehyde-modified glass substrates using a standard array spotter. After placing in an incubator at 25 °C for 2 h, the glass substrate was irradiated with UV-light for 4 min to induce photopolymerization. The glass substrate was heated at 100 °C for 10 s and placed under a fluorescent microscope. As shown in Figure 1, no images were observed before the thermal stress (Figure 1A) while arrayed fluorescence images were produced upon heating (Figure 1B).
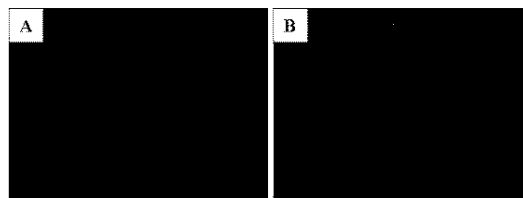

Figure 1. Images observed under a fluorescent microscope with immobilized PDAs before (A) and after (B) thermal stress (100 °C, 10 s). The dot sizes are 250 μm.

Since the immobilized PDA liposomes generate fluorescence on the thermal stress, we next focused on the feasibility of fluorescence change upon ligand-receptor interactions. For this purpose, cyclodextrins were selected. Cyclodextrins (CDs) are intriguing molecules because they form inclusion complexes with a variety of substrates. In addition, different binding specificities of α–, β–, and γ– CDs make these cyclic carbohydrates attractive model systems for studying ligand-receptor interactions. Previously, we reported CD-induced color changes in a polymerized diacetylene LS film. We observed that α-CDs was superior to β- or γ-CD in terms of capability of disrupting the closely packed PDA assembly. If CD can induce blue-to-red color change of PDA film, the fluorescence change is also expected (Figure 2).
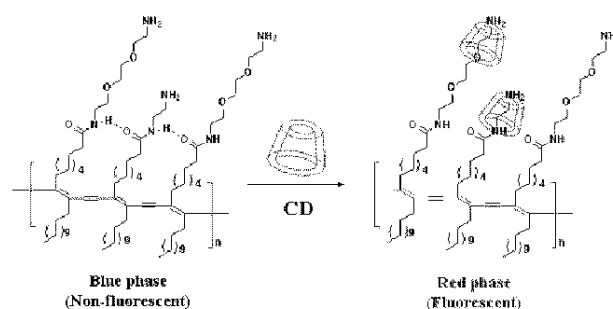

Figure 2. A schematic representation of molecular interaction between polydiacetylene supramolecules and cyclodextrin.

In order to test above possibility, the immobilized PDA arrays prepared using a microarray spotter were incubated for 1 h in solutions containing 30 mM of cyclodextrins or a linear carbohydrate, maltoheptaose (MH) (β-CD was not tested due to the poor solubility). Since the immobilized PDAs contain terminal amine moieties, the effect of ionic interaction with polyacrylic acid (PAA) was also investigated.

Figure 3A shows the fluorescence profiles observed with the immobilized PDA liposomes. As can be seen from the fluorescence profiles, PDAs exposed to α-CD solution results in the generation of red-colored arrayed images (panel a) while those treated with γ-CD (panel b) as well as the linear carbohydrate, maltoheptaose (panel d) reveal virtually no fluorescence. These observations demonstrate that interaction between PDAs and α-CD is superior to those with other carbohydrates. The generation fluorescence by heating the γ-CD (panel c) and MH (panel e)-treated PDAs confirms PDAs are successfully immobilized. Ionic interaction with PAA also caused perturbation of PDAs (panel f). Figure 3B and 3C present visible spectral changes and photographs of PDA liposome solutions in the presence of carbohydrates and PAA. The solutions containing only α-CD and PAA undergo color transition and these results are in good agreement with those described in the fluorescence profiles. Interestingly, the ability of α-CD for disrupting the ordered structure of PDAs was found to be superior to PAA in solution than on the solid substrates. This is presumably due to the difference between three dimensional interactions in solution and two dimensional interaction on solid substrates. The stress induced by long polymer chains on the solid substrate could be more effective than in solution.
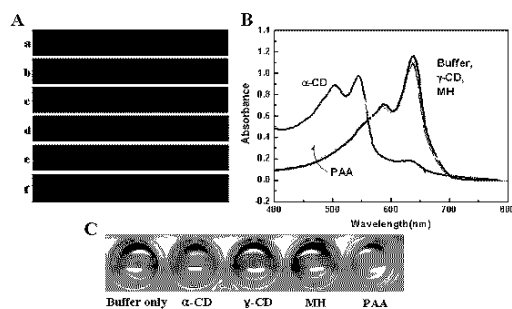
Figure 4. A: Microarray-based fluorescence profiles of PDA-immobilized glass substrates after treatment with α-CD (panel a), γ-CD (panel b), γ-CD and heating (panel c), MH (panel d), MH and heating (panel e), and PAA (panel f); B: UV-visible spectra of PDA liposome solutions in the presence of carbohydrates and PAA; C: Photographs of liposome solutions as in B.

As a next step to demonstrate the feasibility for biological chips, we investigated label-free detection of protein-protein interaction using the conjugated diacetylene systems. Three diacetylene lipids, PCDA, PCDA-EDEA-SA-NHS and PCDA-Biotin, were dissolved in 1 ml chloroform by 8.5:1.0:0.5 molar ratio. The PDA mixtures were processed as previously described to make liposomes and reacted with primary antibodies. The solution containing the liposomes was spotted onto avidin-coated glass slides and immobilized by biotin-avidin binding. The liposomes were polymerized by the exposure to UV light. Then, the anti-rabbit secondary antibody solution was dispersed and hybridized on the glass slides.

The fluorescence levels of the liposomes 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 (Fig. 5B). It was reported that when PDA liposomes were perturbed, the emission light of the fluorescent liposomes was detected at 580 nm. After the secondary antibodies were reacted with primary antibodies on the liposomes, the liposomes turned to be fluorescent on a red filter (Fig. 5C). The images obtained on two filters were overlapped, suggesting that the liposomes changed their fluorescence property by binding with secondary antibodies. Through heating of the glass slide that gives a strong perturbation to the liposomes, the presence of PDA liposomes was confirmed (Fig. 3D). The experiment was repeated three times and similar results were obtained each time.
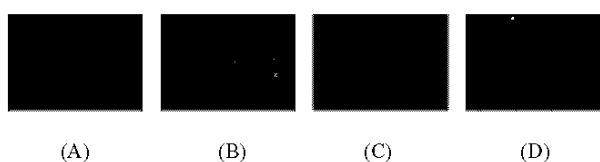

Figugre 5. Fluorescent images of micro-patterned PDA liposomes (A) before the experiment, (B) after incubated with FITC-labeled anti-rabbit secondary antibodies monitored on a green filter (515 nm) and (C) on a red filter (580 nm), and (D) after 5 min heating. (A) and (D) images were taken on a red filter,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

To prove that the binding between PDA liposomes and secondary antibodies was mediated by primary antibodies, two control experiments were carried out. The first was a reaction between the liposomes without primary antibodies and FITC-labeled anti-rabbit secondary antibodies. When the liposomes without primary antibodies were reacted with the anti-rabbit secondary antibodies, no fluorescence was observed on the glass slide (Fig. 6A), suggesting that no external perturbation was made on the liposomes. Without primary antibodies, the interactions between the liposomes and the secondary antibodies were not made. By heating the glass slide, the presence of the liposomes was also confirmed (Fig. 6B).
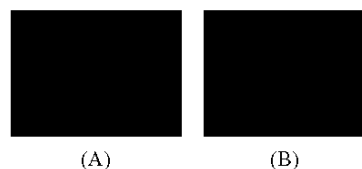

Figure 6. Fluorescent images of micro-patterned PDA liposomes without primary antibodies (A) after incubation with FITC-labeled anti-rabbit secondary antibodies on a red filter (580 nm) and (B) after 5 min heating (red filter).

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 (Fig. 7). The two control experiments proved that no fluorescence level change was made when the primary antibodies on the liposomes did not interact with secondary ones in the solution.
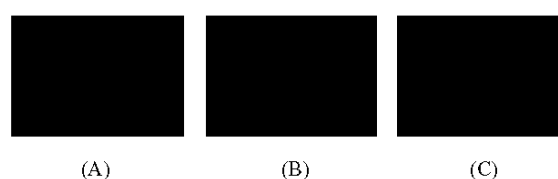

Figure 7. Fluorescent images of micro-patterned PDA liposomes (A) after incubation with FITC-labeled anti-mouse secondary antibodies on a green filter (515 nm) and (B) on a red filter (580 nm) and (C) after 5 min heating (red filter).

Conclusions

We have described a new approach for the PDA-based fluorescent sensor chip system which is compatible with conventional microarray technologies. The specific ligand-receptor interaction allowed generation of patterned fluorescence profiles. As a demonstration, micro-patterned PDA liposomes were proved as a biochip to detect a protein-protein interaction. The liposomes were conjugated with primary antibodies and immobilized on glass slides as an array form. The fluorescence level of the PDA liposomes was changed by protein-protein interactions between primary and secondary antibodies. This result proves that the micro-patterned PDA liposomes are useful to detect protein level interactions and can serve as a novel substrate to develop a protein chip. Combining with modern array-based sensing technologies, the stress-induced self-fluorescent nature of the PDAs should be very useful in the development of label-free chemical/biological sensor systems.

References

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