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A Polydiacetylene-Based Fluorescent Sensor Chip

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Since the first report of the colorimetric detection of the influenza virus by using a polydiacetylene (PDA) film,[1] the development of efficient sensory systems based on PDAs continues to be of great interest.[2] The unique applicability of PDAs as chemosensors derives from the fact that these supramolecules undergo a blue to red visible color change in response to a variety of environmental perturbations, such as temperature,[2a,j] pH,[2k] and ligand−receptor interactions.[2d−h] Consequently, sensing by almost all of the polydiacetylene-based chemosensors reported thus far has been monitored by visible spectroscopy.

It has been known for some time that "blue-phase" polydiacetylenes are nonfluorescent, while their "red-phase" counterparts fluoresce.[3] Despite this property, little effort has been devoted to developing the fluorescence signaling features of polydiacetylene sensors.[3] Recently, we reported a new strategy for patterned fluorescent imaging with PDAs that employs a microcontact printing technique.[4] In that study, we demonstrated that immobilized PDAs undergo transitions from nonfluorescent to fluorescent states upon thermal stress, and that the fluorescence images are readily observed by using fluorescence microscopy. We felt that it would be intriguing to investigate whether this fluorescence change could be used to signal specific ligand−receptor interactions. If this were the case, PDA systems would become useful sensor matrices. In this communication, we report the results of an investigation that has led to the development of an immobilized polydiacetylene conjugated sensor system that is based on fluorescence changes.

In contrast to conventional PDA LB/LS films or vesicle solutions, immobilized PDAs are more advantageous in terms of signal intensity and/or applicability to miniaturized arrayed sensor systems. Our initial efforts focused on the generation of immobilized PDA vesicles on glass substrates. For this purpose, we have used the 10,12-pentacosadiynoic acid (PCDA)-derived diacetylene monomers, PCDA−EDEA and PCDA−EDA, both of which contain terminal amine groups (Figure 1).

A mixture of PCDA−EDEA and PCDA−EDA (1:1, molar ratio) was used in the routine procedure for forming self-assembled diacetylene vesicles in aqueous solution on an aldehyde-modified glass substrate (37 °C for 4 h, see Supporting Information). Immobilizations with various ratios of the two PDA monomers showed that a 1:1 mixture is optimal.

Although the immobilization process can be monitored by visible spectroscopy, evaluation of the patterned images was best carried out by using fluorescence changes (see Supporting Information).

In order for the immobilized PDAs to be applicable to an arrayed sensor system, a procedure for generating well patterned fluorescence images is required. Accordingly, the glass substrate with immobilized diacetylene vesicles was irradiated through a photomask with UV light for 4 min. This process leads to photopolymerization of the immobilized diacetylene vesicles in the exposed areas. The glass substrate was then heated at 100 °C for 10 s to induce the blue-to-red color transition of the polydiacetylene molecules. Since in its red-phase polydiacetylene is strongly fluorescent, it is possible to observe the patterned images generated in the photolithography process by using fluorescence. In Figure 2, the patterned fluorescence images observed under a fluorescent microscope are displayed (red, bright corresponds to areas exposed to UV light). The clear images obtained by this methodology demonstrate that the immobilization process is successful.

We next focused our attention on assessing the feasibility of using a microarray spotter to generate a patterned array of a PDA image that would be more versatile and practical in constructing chip sensor systems. A vesicle solution, prepared with a 1:1 mixture of diacetylenic lipid monomers, PCDA−EDEA and PCDA−EDA, was applied to aldehyde-modified glass substrates by using a standard array spotter. After being incubated at 25 °C for 2 h, the glass substrate was sequentially irradiated with UV light for 4 min and heated at 100 °C for 10 s.

As shown in Figure 3, no fluorescence images were observed prior to the heating step (Figure 3A), while a nicely arrayed set of fluorescence images were produced following thermal treatment (Figure 3B). The above results show that a spotted PDA vesicle array becomes fluorescent when subjected to thermal stress.

In the next phase of this investigation, we evaluated the possibility that this fluorescence change could be promoted by using ligand−receptor interactions. Cyclodextrins (CDs) are intriguing molecules because they form inclusion complexes with a wide variety of substrates. In addition, the different binding specificities of α-, β-, and γ-CDs make these cyclic carbohydrates attractive model systems for studying ligand−receptor interactions.[5] Previously, we discovered that CDs induce color changes in a polymerized diacetylene LS film.[6] In addition, we observed that α-CD is superior to β-CD or γ-CD in its ability to disrupt closely packed PDA assemblies. If CDs are capable of promoting the blue-to-red color transition of PDA films, then they might be able to induce the corresponding fluorescence change (Figure 4).

To test this proposal, four immobilized PDA arrays, prepared by using a microarray spotter, were exposed for 1 h to independent solutions (80 μL, HYB chamber) containing 30 mM α-CD, γ-CD, the linear carbohydrate, maltoheptaose (MH), and poly(acrylic acid) (PAA). The amount of analyte solution can be reduced to less than 10 μL when a cover glass was used, which is one of the merits of employing a miniaturized assay system (see Supporting Information). β-CD was not used due to its poor solubility. In addition, since immobilized PDAs contain terminal amine moieties, interactions between amines and carboxylic acids were probed by using PAA.

In Figure 5A, the fluorescence profiles for the immobilized PDA vesicle arrays are shown. The PDA vesicle incubated with a solution

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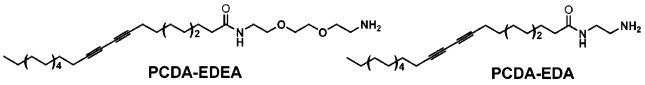

Figure 1. Diacetylene monomers investigated for PDA sensor chip.

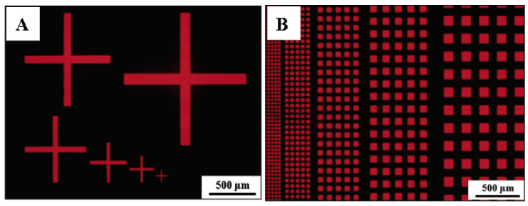

Figure 2. Patterned fluorescent images (A and B) obtained from photomasked irradiation of immobilized diacetylene vesicles, prepared with PCDA−EDA/PCDA−EDA (1:1, molar ratio) on the aldehyde-modified glass substrate.

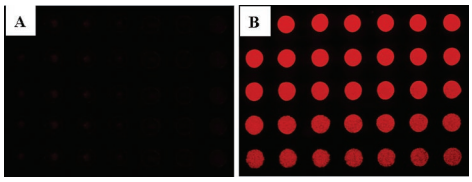

Figure 3. Fluorescent microscope images of microspotted, immobilized, and irradiated PDAs before (A) and after (B) thermal stress (100 °C, 10 s). The dots are 250 μm in diameter.

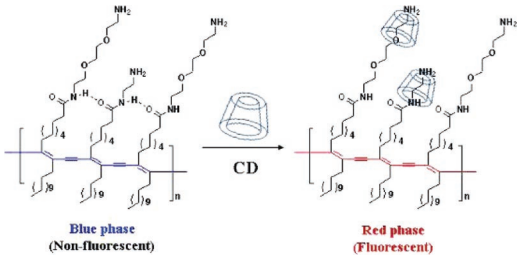

Figure 4. A schematic representation of the interaction between polydiacetylene and cyclodextrin.

of α-CD gives rise to a red-colored fluorescent array image (panel a), while those treated with γ-CD (panel b) and the linear carbohydrate, maltoheptaose (panel d), are blue and nonfluorescent. These observations demonstrate that the interaction between PDAs and α-CD is much stronger than that with other carbohydrates. That fluorescence can be generated by heating the γ-CD and MH-treated PDAs (panels c and e, respectively) confirms that the PDAs were successfully immobilized in these cases. Ionic interaction between PAA and the PDAs also causes a fluorescence perturbation (panel f). The fluorescence spot intensities promoted by α-CD or PAA are concentration dependent (Supporting Information).

The visible spectroscopic changes and photographs of PDA vesicle solutions in the presence of carbohydrates and PAA are shown in Figure 5B and C, respectively. Only PDAs incubated with solutions containing α-CD and PAA undergo color transition. These results are in good agreement with those arising from fluorescence monitoring. Interestingly, the ability of α-CD to disrupt the ordered structures of PDAs is superior to that of PAA in solution rather than on solid substrates. This is presumably due to the differences

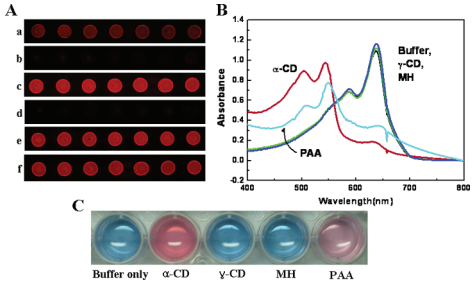

Figure 5. (A) Microarray-based fluorescence profiles of PDA-immobilized glass substrates after treatment with α-CD (panel a), γ-CD (panel b), γ-CD and heating (panel c), MH (panel d), MH and heating (panel e), and PAA (panel f). (B) Visible spectra of PDA vesicle solutions in the presence of carbohydrates and PAA. (C) Photographs of vesicle solutions as in B.

between three-dimensional interactions occurring in solution versus two-dimensional interactions on solid surfaces. The stress induced by long polymer chains could be more effective on solid surfaces than in solution.

In summary, we have developed a new approach for the construction of PDA-based fluorescent chemosensor systems which is compatible with conventional microarray technologies. By using specific ligand−receptor interaction occurring between cyclodextrins and PDA vesicles, patterned fluorescence profiles were generated. Combining this methodology with modern array-based sensing technologies, the stress-induced self-fluorescent nature of the PDAs should be widely applicable to the development of new and interesting PDA-based chemosensor systems.

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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