Immobilization of Polydiacetylene Sensor on Solid Substrate

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ABSTRACT

Immobilized polydiacetylene vesicles on solid substrates were successfully prepared by utilizing imine formation or Diels-Alder reaction. Incubation of an aldehyde-modified glass substrate in an aqueous solution containing self-assembled diacetylene vesicles allowed efficient formation of immobilized polymer vesicles. In addition, preparation of immobilized polydiacetylene vesicles on solid substrate was achieved, for the first time, using the Diels-Alder reaction with diacetylene monomers having maleimido headgroups and a furan-modified glass substrate. Patterned fluorescent images were obtained with immobilized vesicles by selsctive irradiation through a photomask followed by heating the glass substrate at 100 ^{o}C.

Keywords: immobilization, polydiacetylene, vesicles, fluorescent images

1 INTRODUCTION

Recently, the development of efficient sensors utilizing conjugated polymers as sensing matrices has gained much attention among many researchers[1]. Especially, polydiacetylene (PDA)-based sensors for the detection of biologically important species have been intensively investigated due to the unique color changing properties upon stimulation[2]. The advantage of the nanostructured polydiacetylenes as biosensors comes from the fact that visible color change from blue to red occur in response to a variety of environmental perturbations, such as temperature, pH, and ligand-receptor interactions.

The vast majority of polydiacetylene-based sensors reported to date have been prepared in the form of liposomes in aqueous solutions or thin films on solid supports using Langmuir-Blodgett or Langmuir-Schaefer methods. Recently, immobilization of polydiacetylene vesicles onto gold film was reported[3]. Very recently, we reported immobilization of polydiacetylene vesicles on aldehyde substrate via imine formation[4]. Here, we present immobilization and patterned fluorescence images of polydiacetylenes on solid substrate using two different strategies.

2 EXPERIMENTAL

2.1 Preparation of diacetylene monomers

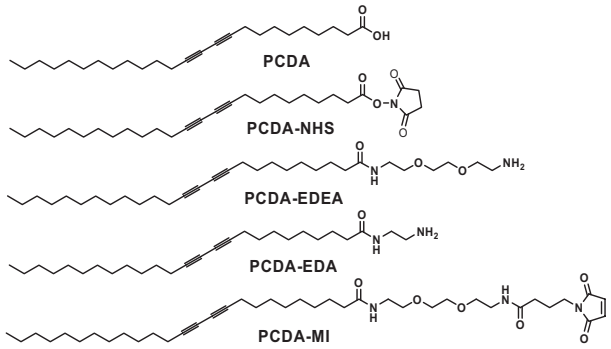


Figure 1: Diacetylene monomers used in this study

PCDA-NHS

To a solution of PCDA (4.00 g, 10.68 mmol, GFS Chemicals) in CH_2Cl_2 (40 mL) was added N-hydroxysuccinimide (NHS) (1.35 g, 11.75 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDC) (2.46 g, 12.82 mmol). The solution was allowed to stir at ambient temperature for 3 h followed by removing the solvent *in vacuo*. The residue was extracted with ethyl acetate and water. The organic layer was dried with $MgSO_4$ and the solvent was removed *in vacuo* to give a white powder (4.83 g, Y=96%)

PCDA-EDEA

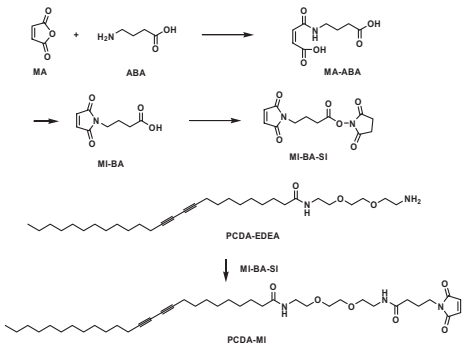
To a solution of PCDA-NHS (5.03 g, 10.68 mmol) in CH_2Cl_2 (200 mL) was added a solution of EDEA (15.83 g, 106.80 mmol) in CH_2Cl_2 (100 mL). The mixture was allowed to stir at ambient temperature for 3 h followed by removing the solvent *in vacuo* much as possible and poured into ethyl acetate. After the precipitate was filtered off, the filtrate was concentrated *in vacuo* to give a white powder (4.09 g, Y=76%).

PCDA-EDA

To a solution of PCDA-NHS (2 g, 4.24 mmol) in CH_2Cl_2 (80 mL) was added a solution of EDA (5.10 g, 84.80 mmol) in CH_2Cl_2 (100 mL). The mixture was allowed to stir at ambient temperature for 6 h followed by removing the solvent *in vacuo* much as possible and poured into ethyl acetate. After the precipitate was filtered off, the filtrate was concentrated *in vacuo* to give a white powder (1.14 g, Y= 65 %).

PCDA- MI

Coupling of maleic anhydride with aminobutyric acid yielded 4-maleimidobutanoic acid (MI-BA) in two steps. The acid MI-BA was converted to the activated ester MI-BA-SI in the presence of N-hydroxysuccinimide. The desired diacetylene monomer PCDA-MI was obtained by coupling the activated ester MI-BI-SI with the amine-terminated diacetylene lipid PCDA-EDEA.



2.2 Preparation of liposome

Diacetylene monomer was dissolved in chloroform in a test tube. The solvent was evaporated by a stream of a N_2 gas and buffer solution was added to the test tube to give desired concentration of a lipid (1 mM). The resultant suspension was sonicated for 15 min at a temperature of around 80 ℃. Following sonication, the solution was filtered to remove dispersed lipid aggregates by using a 0.8 ㎛ filter and cooled at 4 ℃ for overnight. Polymerized diacetylene liposomes are prepared by UV irradiation (1 mW/cm^2) with 254 nm.

2.3 Preparation of glass substrate

Amene/aldehyde-modified glass was purchased from CEL Associate. The furan-modified glass was obtained by incubation of amine-modified glass in a solution containing FA-SA-NHS (5 mM in CH_2Cl_2) for 1day.

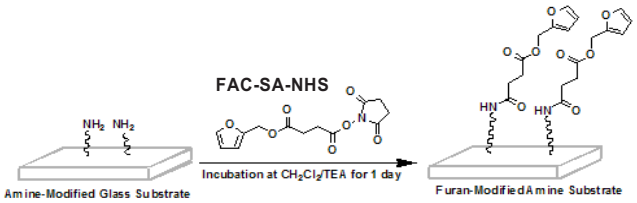


Figure 2: Furan-modification of an amine glass substrate

2.4 Immobilization of polydiacetylene liposome on glass substrate

Imine formation : an aldehyde-modified glass was placed in the diacetylene liposome (PCDA-EDEA : PCDA-EDA = 50 : 50 mol%) for 1day. The glass was sonicated to remove unreacted liposome for 1min and further washed with excess deionized water and dried under a stream of nitrogen.

Diels-Alder reacation : a furan-modified glass was placed in the diacetylene liposome (PCDA-MI : PCDA-EDEA = 50 : 50 mol%) for 1day. The glass was sonicated to remove unreacted liposome for 1min and further washed with excess deionized water and dried under a stream of nitrogen.

2.5 Fabrication of fine fluorescent patterned images

A photolithographic method shown in Figure 3 was employed for patterned fluorescent images.

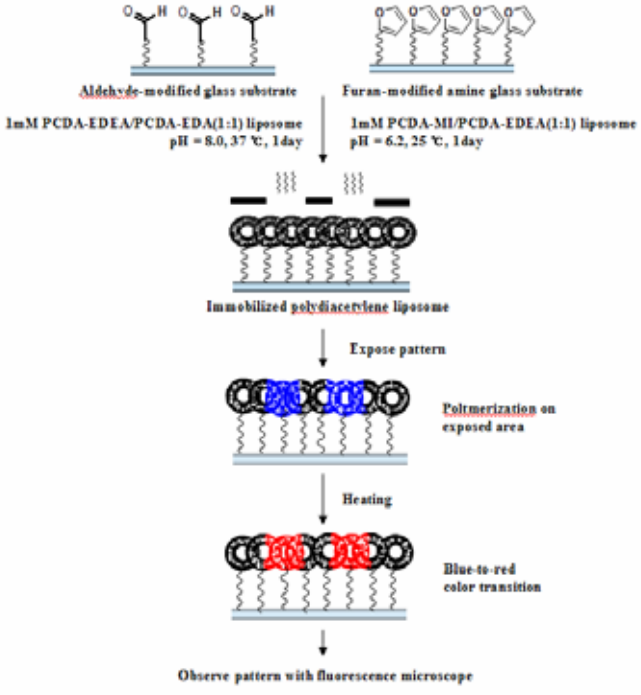


Figure 3: Procedures for patterned fluorescence images.

3 RESULTS AND DISCUSSION

3.1 Immobilization by imine formation

In order to generate immobilized polydiacetylenes on the solid substrate, amine-terminated diacetylene monomers PCDA-EDEA and PCDA-EDA were prepared. A blue-colored and stable polydiacetylene vesicle solution was obtained with PCDA-EDEA when the monomer was subjected to the routine procedures for polydiacetylene vesicle formation in aqueous solution. On the contrary to PCDA-EDEA, the diacetylene monomer PCDA-EDA was converted to only unstable self-assembled diacetylenes which eventually led to solid aggregates. Immobilization on the aldehyde substarte was attempted with self-assembled diacetylene liposomes prepared from various ratios of monomers between PCDA-EDEA and PCDA-EDA. We found best results were obtained with liposomes prepared with a 1:1 mixture of the two monomers.

Figure 4 shows visible spectra after irradiation with UV light for 10 min of the glass substrates immobilized with diacetylene vesicles. It clearly demonstrates that immobilization on the aldehyde substrate (Figure 4A) is more effective than that on a unmodified clean glass substrate (Figure 4B). The control experiment shows that non-specific physical adsorption of the liposomes is negligible.

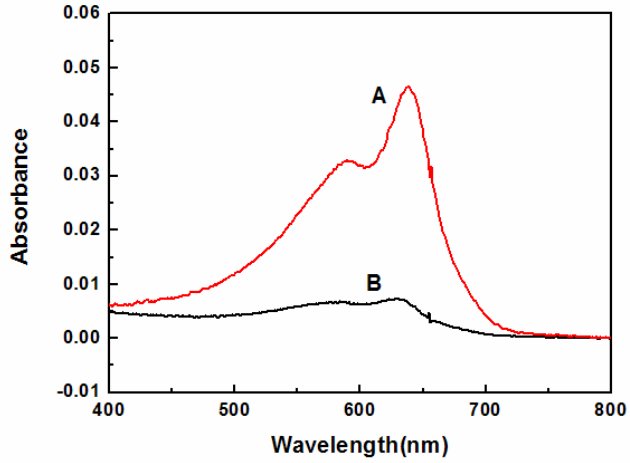


Figure 4: Visible spectra of the immobilized glass substrate after irradiation with UV-light (254 nm, 1mWcm^{-2}) for 10 min. (A: with aldehyde-modified glass substrate, B: with unmodified glass substrate)

Next phase of current investigation focused on the generation of patterned fluorescent images with immobilized polydiacetylenes. For this purpose, a photolithographic method was employed. Accordingly, an aldehyde-modified glass substrate immobilized with diacetylene liposomes derived from PCDA-EDEA and PCDA-EDA (1:1 mixture) was irradiated with UV light for 2 min through a photomask. This process would induce photopolymerization of the immobilized diacetylene vesicles in the exposed areas. The polydiacetylene vesicle-immobilized glass substrate was then heated at 100 ^{o}C for 10 sec to induce the blue-to-red color shift of the polydiacetylene molecules. Since polydiacetylene in the blue phase is nonfluorescent while it is strongly fluorescent in the red phase, it should be possible to observe the patterned fluorescence images. Figure 5 shows patterned fluorescence images observed under a fluorescent microscope. The red (bright) areas are exposed areas with UV light.

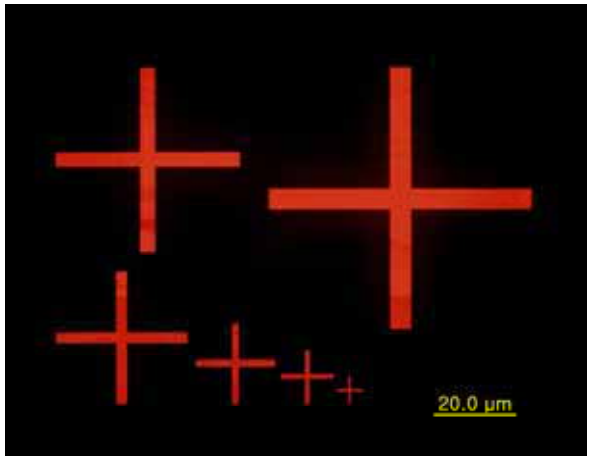


Figure 5: Patterned fluorescent images obtained with PCDA-EDEA/PCDA-EDA (1:1 w/w) as described in the text.

3.2 Immobilization by Diels-Alder reaction

A Diels-Alder reaction is a cycloaddition reaction between a diene and a dienophile. The advantages of bioconjugation utilizing the Diels-Alder reaction are 1) showing little effect of the pH of the reaction medium on the reaction rate, 2) producing no by-products, 3) having rate accelerating effect by hydrophobic interaction between diene and dienophile in aqueous solution. Accordingly, efficient bioconjugation of saccharides to protein and peptides to solid substrates have been reported[5].

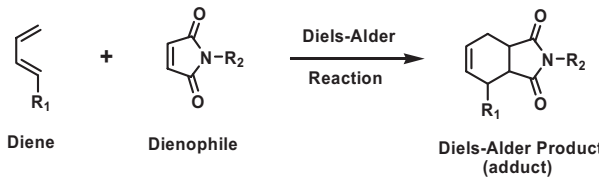


Figure 6: The Diels-Alder reaction between a diene and a dienophile.

Since the bioconjugation using the Diels-Alder reaction has attractive nature, we felt it would be useful if we could immobilize polydiacetylene vesicles using the same approach. For this purpose, we have the modified glass substrate with furan moieties which can form Diels-Alder

adducts with polydiacetylene vesicles. The diacetylene monomer PCDA-MI was designed so that the terminal maleimido group can form adduct with the furan group.

In order to obtain immobilized polydiacetylene vesicles via the Diels-Alder reaction, the furan-modified solid substrate was incubated in the liposome solution prepared with a mixture of diacetylene monomers PCDA-MI and PCDA-EDEA (1:1 w/w). The comonomer PCDA-EDEA was introduced to avoid dense packing of the maleimido moieties on the surface of the vesicles which might have bad effect on the immobilization of the vesicles.

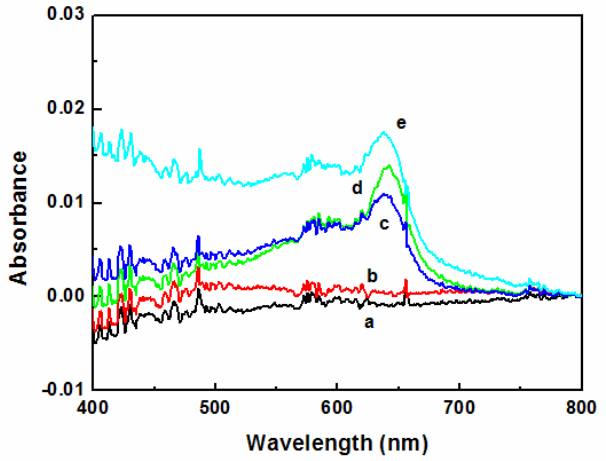


Figure 7: Visible spectra of furan-modified amine glass substrates (a, b and c) and of amine-modified glass substrates (d and e) after immobilizing vesicles prepared with a) PCDA-MI , b) PCDA-EDEA, c) 1:1 mixture of PCDA-MI and PCDA-EDEA, d) PCDA-MI, and e) 1:1 mixture of PCDA-MI and PCDA-EDEA.

As can be seen in Figure 7, immobilization of polydiacetylene vesicles prepared with PCDA-MI alone on the furan-modified solid substrate was failed (Figure 7, a). This is presumably due to the lack of the space needed to form adducts by the Diels-Alder reaction. Immobilization of the vesicles prepared with 100% PCDA-EDEA was not observed (Figure 9, b), demonstrating amine moieties on the vesicles do not interact with furan groups on the solid substrates. Interestingly, the polydiacetylene vesicles prepared with PCDA-MI was successfully immobilized on the amine-modified glass substrate. We believe this could be due to covalent bond formation between the double bond of the amido group and the free amine on the solid support.

In order to confirm the immobilization via Diels-Alder reaction is effective, generation of patterned fluorescent images on solid substrate was attempted. Thus, the furan modified glass substrate was incubated in a diacetylene liposome solution. After immobilization, the solid substrate was irradiated with UV light followed by heating at 100 ^{o}C. As can be seen in Figure 8, finely resolved patterned fluorescent images were obtained.

4 CONCLUSION

We have prepared diacetylene monomers and immobilized the resulting self-assembled diacetylene liposomes onto the glass substrates via imine formation or by Diels-Alder reaction. Both methods were found to be effective for the immobilized polydiacetylene vesicles. Fine fluorescent images were obtained when the immobilized liposomes were irradiated with UV light through a photomask followed by heating the glass substrate at 100℃. The results described in this study should be potentially useful for fabricating stable liposome array sensor systems.

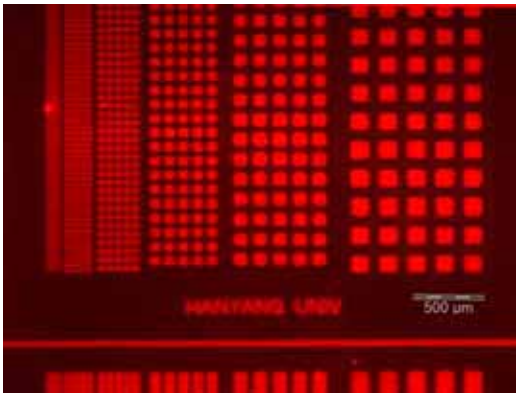


Figure 8: Patterned fluorescent images obtained by sequential immobilization by DIels-Alder reaction, selective irradiation and heating of the glass substrate as described in the text.

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