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A nanoscale vesicular polydiacetylene sensor for organic amines by fluorescence recovery

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Abstract

We report a nanoscale lipid membrane-based sensor of conjugated polydiacetylene (PDA) vesicles for fluorescence detection of organic amines. The vesicle sensor was constructed by incorporation of a BODIPY fluorescent dye into the PDA vesicles. The fluorescent properties of the resulting vesicles can be manipulated by adjusting lipid components, and are controlled by environmental and solution conditions. The fluorescence of the BODIPY dye was significantly quenched in the polymerization of diacetylene lipid vesicles by a UV irradiation process. However, it was sufficiently recovered by external stimuli such as a hike of solution pH. The fluorescence recovery process was reversible, and a decrease in solution pH resulted in repeated quenching. The reported system transforms an external stimulus into a large fluorescence intensity change, demonstrating great potential in developing new signal reporting method for biosensor design. The quench-recovery phenomenon of the BODIPY–PDA is believed to be related to the energy transfer between the dye and the PDA conjugate backbone. The vesicle sensor was applied for detecting an organic amine, triethylamine (TEA) and a large linear relationship was obtained between the increase in fluorescence intensity and the concentrations of TEA. The detection limit of TEA by vesicle sensors using fluorescence recovery was found to be 10 μM.

Keywords: Polydiacetylene; Fluorescence recovery; Vesicle sensor

1. Introduction

Fluorescence spectroscopy is a well-established and widely used technique in various fields of analytical chemistry. In conventional fluorescence detection, either target molecules or their recognition partners are labeled with a fluorescence dye of high quantum efficiency, as many biomolecules lack in intrinsic fluorescence or are too weak to be detected by fluorescence spectrophotometer. However, dye labeling is a time-consuming process. In addition, it may affect the biological properties, such as recognition, binding and catalytical functions of proteins. There exist tremendous interests in developing "smart" fluorescent sensing materials that can respond to analytes directly. Conjugated polymer-based sensors appear to be highly promising for the self-amplifying, direct fluorescence detection [1]. Swager et al. reported a fluorescence chemical sensor for TNT detection, in which the rigid three-dimensional pentiptycene moieties were incorporated into conjugated polymer chain to enhance the fluorescence quantum yield. Strong electron acceptors, such as explosive TNT bind to the cavities of the rigid scaffolds by electrostatic interaction, and the electron transfer from the excited polymer to TNT molecules leads to the quenching of the excited polymer [2]. Although elegant in design, these materials usually involve complex organic synthesis. In addition, many of these polymers rely on fluorescence quenching or wavelength shift. A more desirable detecting method is direct, positive measurement as demonstrated in molecular beacon, where fluorescence increment is directly proportional to the concentration of analytes [3]. Leclerc and coworkers developed fluorescent polythiophenes to detect DNAs. They showed that the polymer fluorescence was quenched by the conformation change triggered by electrostatic interactions with oligos but recovered when the complementary binding took place [4,5]. The work by Chen et al. in developing quenching-recovery

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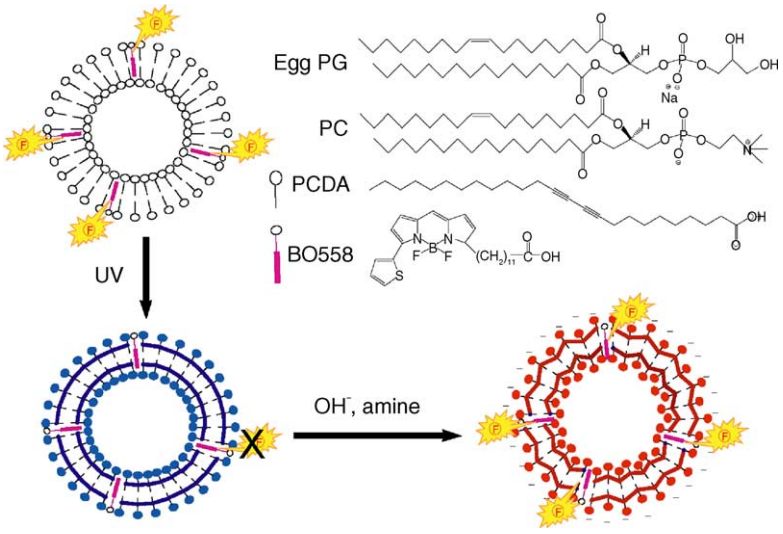

Fig. 1. Molecular structure of the lipids used in the study and a schematic illustration of the vesicle sensor.

fluorescence sensor involving methylviologen was another example [6]. Recently, Mallik and coworkers reported a Lanthanide ion fluorescence sensor for protein analysis [7]. The mechanism involves water molecules bound to Eu^{3+} ions that quench their luminescence via weak vibronic coupling with the vibrational states of the O–H oscillators. After the water molecules were replaced by protein, the luminescence lifetime and intensity of the lanthanide ion were enhanced, providing a positive fluorescence signal change.

In this article, we report a nanometer scale vesicle sensor that demonstrates switchable fluorescence quenching property and allows for analyte detection in a direct, positive response fashion. The vesicular sensor is constructed by incorporation of a bleach resistive BODIPY fluorescence dye into polydiacetylene (PDA) vesicles. Fig. 1 showed the molecular structure of the lipids and a schematic for the design. PDA is a conjugated polymer that has been investigated for colorimetric detection of various biological molecules. The conjugated backbone in PDA undergoes a planer to non-planar transition upon perturbation by external stimuli, such as temperature, pH and organic solvent, and exhibits a blue to red color transition [8]. Colorimetric PDA biosensors have been developed for the detection of CT [9], influenza virus [10], *Escherichia coli* [11], epitope [12] and lipopolysaccharides [13]. The intrinsic fluorescence signal of PDA is weak due to low quantum efficiency, and has not been extensively explored for biomolecular detection. We found that by doping the PDA vesicles with a fluorescence dye, the fluorescence property of the vesicles can be effectively manipulated by lipid components and controlled by environmental conditions. The rapid and reversible response of the fluorescence signal to external stimuli provides a new platform for biosensor design.

2. Experimental

2.1. Materials

10,12-Pentacosadiynoic acid (PCDA) was obtained from GFS Chemicals. 4,4-Difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-inda-cene-3-dodecanoic acid (BODIPY 558/568C_{12}, abbreviated as BO558) was purchased from Molecular Probes. Phosphatidylcholine (PC) and L-α-phosphatidylglycerol (Egg PG) lipids were obtained from Advanti Lipids. Triethylamine and other chemicals were from Aldrich and used as received.

2.2. Preparation of vesicles

A chloroform solution containing 1 mg PCDA lipid (2.67×10^{-6} mol) and 0.25% molar ratio of BO558 (3.15×10^{-3} mg) was added into a 4 mL amber vial. The organic solvent was removed with a nitrogen stream, leaving a thin layer of lipids on the bottom of the vial. Three milliliter of D.I. water was added into the vial, and the vial was probe sonicated for 20 min to reach a clear solution. The solution was then put into a refrigerator and incubated at 4 °C for 1 h. In a comparison experiment, saturated PC lipid was mixed with the above solution in chloroform and the molar ratio of PC was made between 10 and 30% as compared with PCDA. For the control experiment, vesicles were prepared using saturated L-α-phosphatidylglycerol lipids (0.89 mg) and BO558 in a molar ratio of 100:0.5.

2.3. Polymerization

Photopolymerization of the lipid vesicles was carried out using an UV cross-linker (254 nm). Samples were pipetted into a 96-well microplate (150 μL in each well) and irradiated at 0.3 J/cm^2 for 1 min to yield a deep blue color.

2.4. Fluorescence measurement

Fluorescence measurement was preformed on a HORIBA FluoroLog spectrofluorometer with a 475 nm excitation wavelength. The emission spectra were collected in the range of 500–650 nm. Six hundred microliter polymerized vesicle solution was transferred into a glass tube cuvette for fluorescence measurement. For pH-controlled experiment, 20 μL of 0.1 M NaOH or 0.1 M HCl was added into the solution to adjust the solution pH. For organic amine test, 50 μL of TEA solution from a concentration range of 500 μM to 20 mM was added into 450 μL vesicle solution in cuvette.

2.5. Particle sizing measurement

Dynamic light scattering (DLS) particle sizing measurements were preformed by a ZetaPALS Zeta Potential Analyzer from Brookhaven Instruments. Vesicle solutions were diluted 5 to 6-fold and the typical count rate was kept at 700–800 kcps. Each diameter value was an average result of continuous measurements in 5 min. At least three measurements were preformed for each solution.

2.6. Transmission electron microscopy (TEM)

The TEM images of vesicle solution were obtained with a Phillips TECNAI12 transmission electron microscope operating at 100 kV. Carbon-coated copper grids were used for the preparation of specimen. The samples were negatively stained with 2% uranyl acetate.

3. Results and discussion

3.1. Switchable fluorescence quenching of BODIPY-doped vesicles

The vesicle sensors were constructed with two lipid components, PCDA and BO558 in a molar ratio of 100:0.25. BO558 is a lipophilic fluorescence dye that can be inserted into the bilayer membrane. BODIPY dye was chosen over other fluorophores because of its strong resistance to photobleaching. UV irradiation, required for photopolymerization of diacetylene, will not cause significant quenching of the dye in the membrane. Fig. 2A shows the fluorescence spectra of BO558-doped vesicles excited at 475 nm under various conditions. Emission occurs at 575 nm, which is slightly higher than the reported value in methanol (568 nm). It is important to note that the fluorescence intensity of the vesicles in unpolymerized vesicle was only about 10% of that of BO558 dye in chloroform, showing a considerable degree of quenching has occurred even before polymerization. After polymerization of the diacetylene lipids with UV, the fluorescence signal was further reduced, decreasing to about only 0.5% of the intensity of the dye in organic solvent (Fig. 2A).

The quenched vesicles were further investigated to examine the effect of solution conditions, in particular basicity, on the fluorescence properties. To our surprise, addition of 20 μL of 0.1 M NaOH into the 600 μL of vesicle solution results in a tremendous recovery of the fluorescence signal. The obtained intensity was not only higher than that after polymerization, it was also almost twice as much as the intensity of the vesicles before polymerization (curve 3 in Fig. 2).

Interestingly, the fluorescence recovery induced by the base is a reversible process. When the vesicles were neutralized by 20 μL of 0.1 M HCl, a substantial decrease in fluorescence was observed (curve 4). At this stage, formation of some degree of precipitation was noticed. Addition of 0.1 M NaOH solution to this solution results in repeated fluorescence recovery, and the obtained intensity was comparable to the first recovery, demonstrating high reproducibility of the process. Fig. 2B is a bar plot showing the comparison of fluorescence intensity at different solution conditions. From the bar chart, a trend of reversible change in fluorescence intensity based on solution basicity is quite obvious.

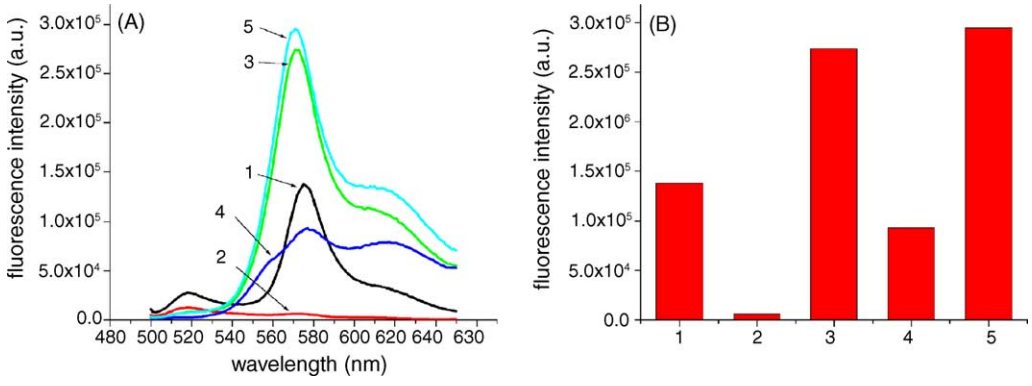

Fig. 2. Fluorescence spectra (A) and intensity bar chart (B) of PDA–BO558 vesicle sensors under different solution conditions. The molar ratio of PCDA and BO558 is 100:0.25. The solution conditions are: (1) before UV irradiation, (2) 1 min UV irradiation, (3) 20 μL 0.1 M NaOH, (4) 20 μL 0.1 M NaOH + 20 μL 0.1 M HCl, and (5) 40 μL 0.1 M NaOH + 20 μL 0.1 M HCl.

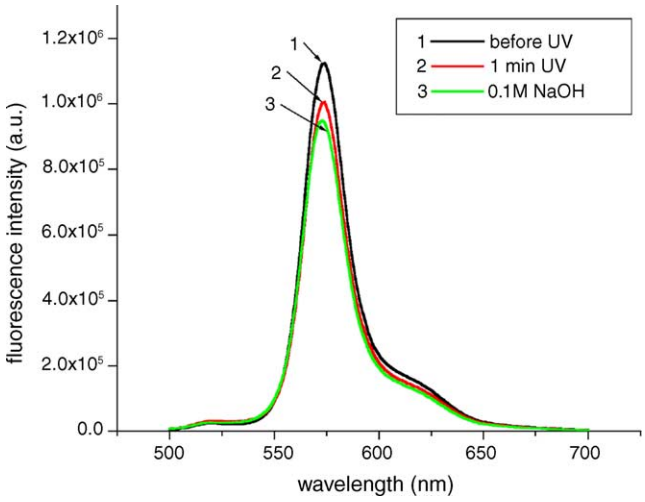

Fig. 3. Fluorescence spectra of Egg PG–BO558 vesicles. The concentration of Egg PG was 0.30 mg/mL and molar ratio of Egg PG and BO558 was 100:0.5. Experimental conditions: (1) before UV irradiation, (2) after 1 min UV irradiation and (3) addition of 50 μL of 0.1 M NaOH.

To understand the role of diacetylene group in the quenching of BODIPY dye and effect of base on the dye as a whole, a comparison experiment was carried out using vesicles prepared by saturated L-α-phosphatidylglycerol lipids (Egg PG, structure shown in Fig. 1), and the results are shown in Fig. 3. High fluorescence intensity was observed for the PG–BO558 vesicle, which is comparable to that of the dye in organic solvent. In addition, no quenching effect was observed after UV irradiation or treatment of NaOH solution. This result clearly suggests that the BODIPY dye in the membrane is insensitive to UV irradiation or the base. The quenching effects observed in the PDA vesicles are highly associated with the conjugated group in the membrane.

Table 1
Results of DLS particle size measurement for BO558 vesicles

	Particle size (nm)
Before UV	113 ± 2
1 min UV	109 ± 2
0.1 M NaOH treated	253 ± 5

3.2. Characterization of PDA–BO588 vesicles

We next characterized fluorescence vesicles by using dynamic light scattering (DLS) and transmission electron microscopy. DLS offers a convenient method to monitor the size change of the vesicles under various conditions. Table 1 shows the results. The PCDA–BO558 vesicles have a relative uniform size distribution, giving an average size of ca. 113 nm in diameter. After 1 min UV irradiation for polymerization, the size is reduced to 109 nm, which is not a significant size change. Despite the covalent linkage formed during polymerization, the tight packing of lipids in diacetylene vesicles apparently leaves very little room for further squeezing. TEM study supports the DLS observation. Fig. 4 shows a TEM image of the polymerized vesicles of PDA–BO558.

After treating with 0.1 M NaOH, the vesicle size increases substantially. It appears that the repulsion force developed between the negative charged headgroups causes the swelling of the vesicles. As fluorescence recovery occurs at high pH, we speculate that two factors might be responsible for the recovery of the fluorescence: distance and conformational change. The repulsive force increases the side chain distance, and possibly the distance between ene-yne conjugate bond and dye, leading to poor energy transfer between the dye and the conjugated system, which reverses the quenching. Conformational change involves a planar to non-planar transition of the polymer backbone, which was known influenced by the

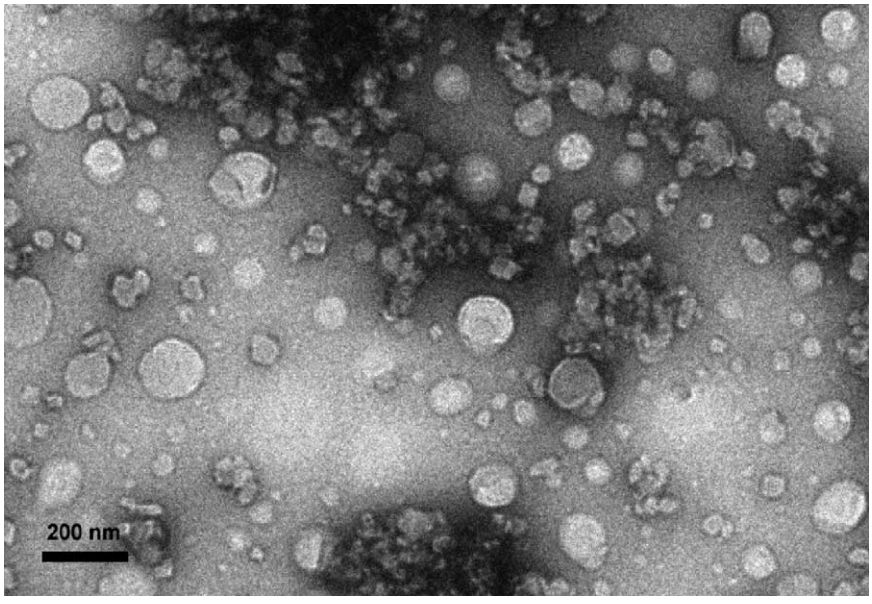

Fig. 4. A TEM image of PDA–BO558 vesicles.

Table 2
Quenching efficiency for PDA–BO558 vesicles with and without PC

Vesicle systems	Quenching efficiency (%)
PDA–BO558	97
PDA–BO558–10%PC	96
PDA–BO558–30%PC	92
PDA–BO558–50%PC	72

increase of the solution pH. However, one must note that this may not likely be the main reason because the planar to non-planar conformation change is typically irreversible but the quenching of fluorescence is recoverable.

We further tested the distance factor by doping phosphatidylcholine (PC) into the BODIPY–PDA vesicles. Different percentage of PC lipids was incorporated into PDA–BO558 vesicles and their fluorescence quenching properties were compared. As showed in Table 2, the quenching became weaker as more PC lipids were incorporated into the PDA vesicles. The quenching efficiency decreases from 97 to 72% after incorporation of 50% of PC. Quenching efficiency was obtained by calculating the percentage of fluorescence intensity left after 1 min UV irradiation as compared with that before polymerization. We speculate that incorporation of PC dilutes the ene-yne conjugates and thus makes the interaction between the dye and polymer backbone difficult.

Admittedly, the mechanism of the reversible fluorescence quenching observed on PDA–BO558 vesicles is not fully understood at this stage. The quenching effect observed in unpolymerized diacetylene vesicles may stem primarily from the interaction of electron-rich triple bonds and the ring in the BODIPY dye. In the polymerized vesicles, the extended π-conjugate backbone plays in important role. PDA has strong absorption at the emission wavelength of BO558. The polymerization lowers the energy gap, leading to more effective energy transfer between the dye and the PDA conjugate backbone. Excitation energy migration between polymer and dye have been reported [14,15]. The energy migration between polymer and dye has been used to improve the optical properties of light-emitting conjugate polymers. The disperse transport of emission energy is thought to be from polymer, which has higher LOMO-HUMO energy separation, to low energy separation molecules. However, in our case, the energy transfer may occur from the excited dye to the conjugate system as the conjugated PDA molecules have an energy band gap of 2.0–2.4 eV, depending on conjugate length and conformation [16,17], while BODIPY has a band gap of 2.04 eV [15].

3.3. Fluorescence detection of organic amines with PDA–BO558 vesicle sensors

To prove the effectiveness of fluorescence recovery reported here for sensing application, we tested the detection of a weak base, triethylamine, with the PDA–BO558 vesicle sensor. A series of TEA solutions were prepared and tested by the vesicle sensor with a mole ratio of 100:0.25 PDA:BO558. Fig. 5 shows the calibration curves for fluorescence response to TEA. The *y*-axis is the increase in fluorescence intensity after TEA is added into the vesicle solution. A linear relationship was obtained for the concentration range of 50 μM to 2 mM, and 10 μM detection limit was measured. The result clearly demonstrates the potential of the system as a chemosensor.

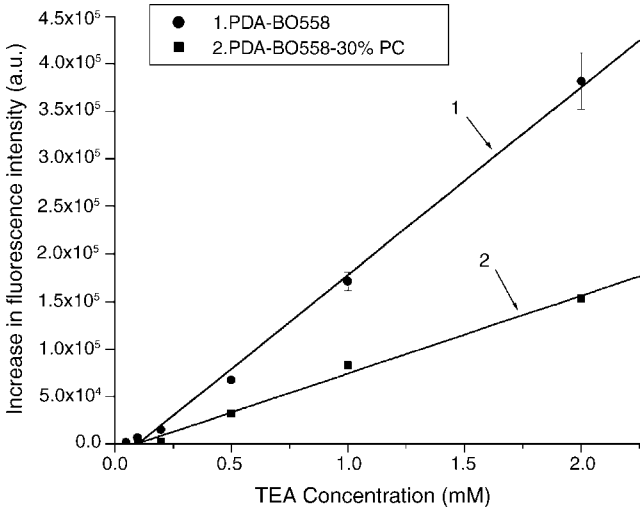

Fig. 5. Calibration curves of fluorescence responses to TEA with two types of vesicle sensors. (1) 100:0.25 PDA:BO558 and (2) 100:30:0.25 PDA:PC:BO558.

The detection sensitivity was further tested with PDA vesicles doped with natural lipid, PC. PC lipids were incorporated into the vesicles for distance factor study, and most importantly, for mimicking biomembrane. The design of biosensor using vesicle sensor would involve incorporation of bio-recognition units, such as GM1 in the case of toxin analysis [9]. We attempted to compare the detection sensitivity of the fluorescence vesicles as a function of bio-relevant component incorporated into the system. From Table 2, the quenching efficiency decreases with incorporation of PC. Fig. 5(2) shows the response curve for TEA with PDA–BO558 doped with 30% of PC. The vesicles showed a linear response to TEA as the undoped PDA–BO558 vesicle sensor, but the detection sensitivity decreased as expected.

Further sensitivity analysis was presented in Table 3, which summarizes the fluorescence recovery for different systems using the same concentration of TEA (200 μM). It is clear that as the percentage of PC increases, the response sensitivity drops. This effect is associated with the lower

Table 3
A comparison of fluorescence recovery towards TEA for different vesicle compositions

Vesicle systems	Fluorescence recovery
PDA–B558/568	7650
PDA–10%PC–B558/568	2400
PDA–30%PC–B558/568	2100
PDA–50%PC–B558/568	100

Recovery values were net fluorescence intensity increase in the presence of 200 μM TEA.

quenching efficiency at high PC content that yields high fluorescence background, as discussed previously. From the table, 30% of PC in the vesicles still offers considerable recovery signal. Indeed, 30% of bio-recognition units in a vesicle sensor is more than sufficient for biosensing [9–11].

4. Conclusions

In conclusion, we have developed a new signal reporting system that consists of conjugated polymer and a fluorescence probe, which enables direct and positive fluorescence detection of an organic amine. The fluorescence intensity of BODIPY dye is quenched to an extremely low level after photopolymerization of the diacetylene lipid assembly, which hosts the lipophilic dye. The fluorescence intensity undergoes tremendous recovery when the organic base is added into the solution to change the solution pH, providing quantitative analysis of the target. The dye-doped vesicle system can convert the environmental factors that disturb the PDA vesicle and its conjugated backbone into changes in fluorescence intensity into changes in fluorescence intensity. The signal transduction is rapid, reversible and highly sensitive, making the system a very promising platform for sensor design. The mechanism of the quenching-recovery phenomenon is not clear but may involve the energy transfer between the dye and conjugated polymers. The PDA–BO558 vesicle sensor shows a linear response to triethylamine and was able to detect 10 μM of TEA. The successful detection of amine proves that this system can be used as a chemosensor. Future work will focus on incorporation of bio-recognition units into the vesicle membrane for detection of a variety of biological significant targets.

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