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Conjugated Biomimetic Polymer Sensors Employing Fluorescence Resonance Energy Transfer

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Abstract

In this study, conjugated biomimetic polymer sensors composed of 10,12-Pentacosadiynoic acid (PCDA) and fluorescent dye BO558 were prepared. Polymerized PCDA vesicles show unique colorimetric and fluorescent changes by external stresses, that is, from blue to red and from non-fluorescent to red-fluorescent. However, the quantum yield of PCDA alone is very low (blue phase: 10^{-4}, red phase: 0.02). Such disadvantage can be overcome via the use of fluorescence resonance energy transfer (FRET) by incorporating fluorescent dye molecules into PCDA vesicles. In this article, we report that heterogeneous PCDA/BO558 vesicle systems can selectively detect the inclusion complex of α-cylcodextrin by using fluorescent spectroscopy. While the fluorescence intensity of BO558 was considerably decreased in polymerized blue-phase PCDA/BO558 vesicles due to energy transfer, the intensity was increased and recovered upon reaction with α-cyclodextrin (CD). On contrary, it was not changed upon exposure to γ-CD, indicating the specific detection performance of the sensor. Such fluorescent changes are considered to result from reduced energy transfer due to a conformational change of the conjugated PCDA polymer backbone by the inclusion complexation of α-CD. These FRET characteristics of PCDA/BO558 vesicles would apply to a wider range of biomolecular recognition events.

Keywords: Polydiacetylene, Fluorescence resonance energy transfer (FRET), Inclusion complex, Biomolecular recognition

Introduction

Specific conjugated polymers induce changes of redox potential, absorption or emission spectrum by internal/external stimuli. Sensors using conjugated polymers have attracted a great deal of interest due to signal amplification, as compared to low-molecular-weight chemosensors[1]. In particular, conjugated diacetylene polymers show unique colorimetric and fluorescent changes by external stresses, that is, from blue to red and from non-fluorescent to red-fluorescent. Color-transition sensors based on biomimetic polydiacetylene (PDA) have been used to detect influenza virus[2], cholera toxin[3], gases[4], α-CD[5-7], etc. In addition to these examples, we recently reported that polydiacetylene-based fluorescent sensors could be employed in the detection of heat[6,7], α-CD[6,7], and protein-protein interactions[8]. However, the fluorescence quantum yield of PDA is very low (blue phase: 10^{-4}, red phase: 0.02)[9]. Meanwhile, conjugated polymers employing FRET have been an important topic in biosensors because these smart materials can be used in a variety of applications, including DNA[10] and protein[11] detection with high sensitivity[12]. In order to overcome low quantum yield, a combination of PDA with fluorescent dye in order to utilize FRET has been reported recently. Cheng *et al*. reported a chemical sensor[13] for detecting organic amines using interactions between the amine and carboxylic acid of a conjugated polymer composed of PDA and 4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-indacene-3-dodecanoic acid (BO558). They also reported fluorescence "turn-on" vesicle sensors[14] with reversible "on-off" switching properties upon pH swing, which were extended to detect a bacterial toxin[15]. Liu *et al*. reported the mechanism of FRET in PDA/BO558 systems upon thermal treatments[16]. They suggested that the increase in fluorescence intensity for the red-phase occured due to changes in energy transfer by the conformational change of PDA polymer backbone upon thermal stress. To the authors' knowledge, a chemical sensor for α-cylcodextrin detection using FRET has not been reported. In this article, we report that heterogeneous PDA/BO558 vesicles can selectively detect the inclusion complex of α-cyclodextrin by the use of fluorescent spectroscopy.

Results and Discussion

Figure 1 shows the molecular structures of PCDA and BO558. It is known that polymerized blue-phase PCDA vesicles have a strong absorption peak at 640 nm, whereas red-phase PCDA vesicles after color change do at 540 nm. It has been reported that the fluorescence emission of BO558 occurs at about 575 nm[16]. The overlap between BO558 emission and PCDA absorption provides the possibility of energy transfer from BO558 to the conjugated polymer backbone.

In an effort to compare the characteristics of the PCDA/BO558 vesicles prepared in the present study with the literatures[14,16], the effects of temperature and pH on fluorescent quenching were investigated. Figure 2 shows the changes in fluorescence intensity of polymerized pure PCDA and heterogeneous PCDA/BO558 vesicles before and after heating. The polymerized blue-phase PCDA vesicles showed no fluorescence (curve 1), whereas the red-phase PCDA vesicles induced by thermal treatment at 110°C had fluorescent emission at about 560 nm (red fluorescence, curve 2). Unpolymerized heterogeneous PCDA/BO558 vesicles exhibited strong fluorescence (curve 3), whereas the blue-phase PCDA/BO558 vesicles polymerized upon UV irradiation for 5 min were reduced in fluorescence intensity (curve 4) to about only 20% of the initial value. This result indicates that the conjugated diacetylene backbone formed by polymerization of PCDA/BO558 vesicles quenches the fluorescence of BO558 incorporated into the PCDA vesicles. When the vesicles were treated by heating at 110°C for 10 min, the fluorescence intensity obviously increased (curve 5) due to reduced energy transfer by the conformational change of PCDA backbone through thermal stress. These results are in good agreement with those reported in the literature[16]. Moreover, the intensity of heterogeneous PCDA/BO558 vesicles was higher than that of the pure red-phase PCDA ones. Therefore, the introduction of BO558 into PCDA vesicles can be more effective in detection performance than the pure PCDA vesicles in the aspect of fluorescence intensity.

Fluorescence spectra of PCDA/BO558 vesicles in response to acid/base addition are shown in Figure 3. The polymerization of PCDA/BO558 vesicles led to a substantial fluorescent decrease of BO558 (curve 2) as compared to unpolymerized vesicles, same as de-

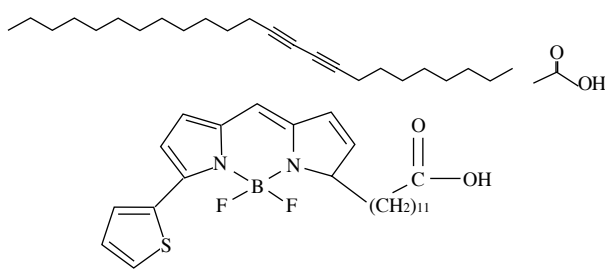

Figure 1. Molecular structures of PCDA (top) and BO558 (bottom).

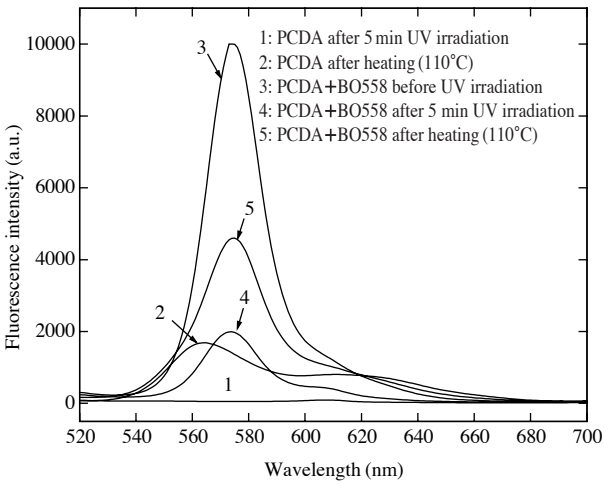

Figure 2. Fluorescence spectra of PCDA and PCDA/BO558 (molar ratio=100 : 0.25) vesicles upon heat treatment: (1) PCDA after 5 min UV irradiation, (2) PCDA after heating at 110°C, (3) PCDA/BO558 before UV irradiation, (4) PCDA/BO558 after 5 min UV irradiation, and (5) PCDA/BO558 after heating at 110°C (The excitation wavelength is 495 nm for all the spectra).

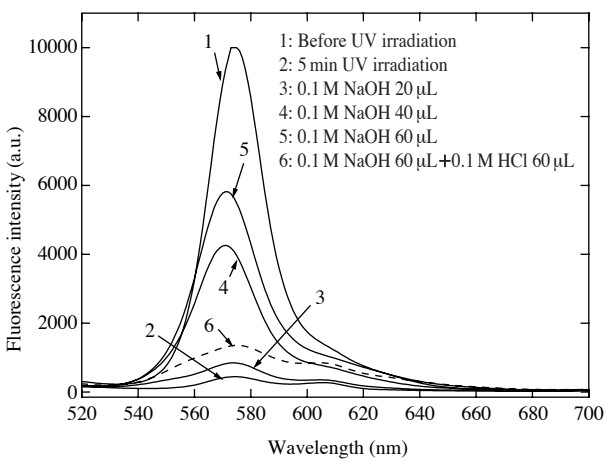

Figure 3. Fluorescence spectra of PCDA/BO558 (molar ratio=100 : 0.25) vesicles upon addition of base and acid: (1) before UV irradiation, (2) after 5 min UV irradiation, additions of (3) 20 μL 0.1 M NaOH, (4) 40 μL 0.1 M NaOH, (5) 60 μL 0.1 M NaOH, and (6) 60 μL 0.1 M HCl to the case of (5).

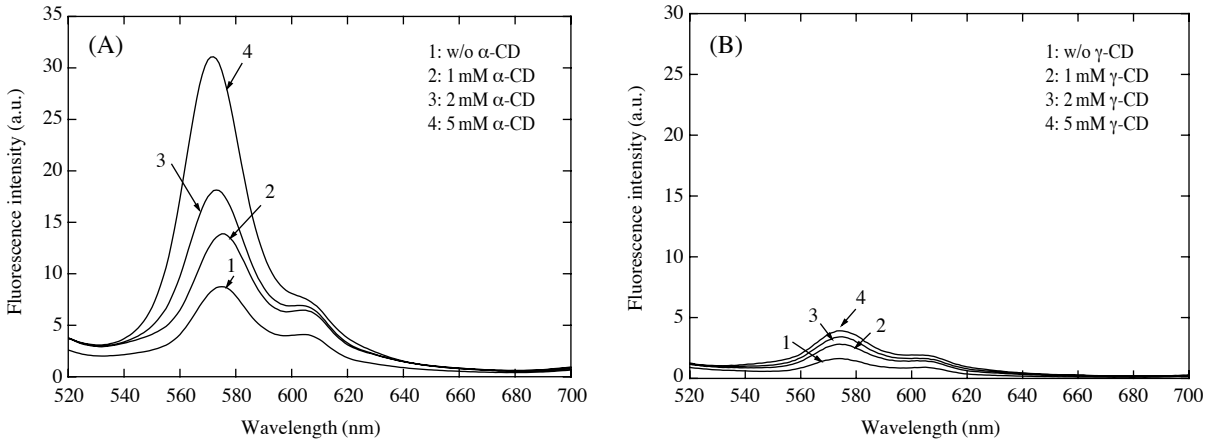

Figure 4. Fluorescence spectra of PCDA/BO558 (molar ratio=100 : 0.25) vesicles after reaction with CDs at different concentrations: (A) α-CD and (B) γ-CD.

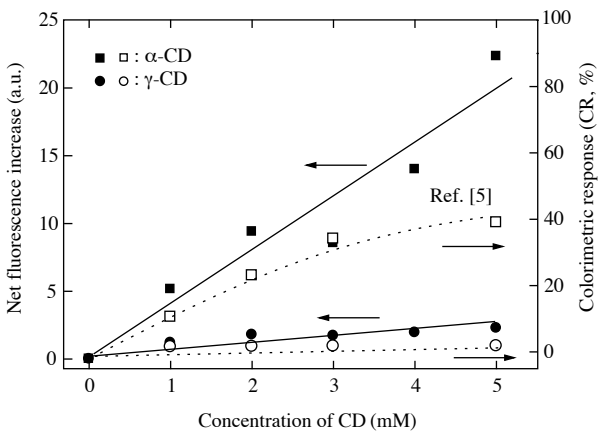

Figure 5. Net fluorescence increase with concentrations of CDs (closed symbols) and comparison with colorimetric responses (open symbols).

picted in Figure 2. When 20 μL of 0.1 M NaOH was added to the vesicle solution, the fluorescence intensity showed a small increase (curve 3). The successive addition of 0.1 M NaOH induced more fluorescence increase (curves 4 and 5). The extent of increase in fluorescence intensity depends on the amount of the basic solution. Meanwhile, the addition of 60 μL of 0.1 M HCl resulted in the quenching of the fluorescence intensity (curve 6). These phenomena are totally reversible as reported in the literature for the case of Gly-PCDA[14]. However, this reversible characteristic contrasts clearly against the irreversible color transition of pure PCDA vesicles upon pH treatments. In such cases, PCDA solution becomes red at high pH, and the red color is maintained in spite of low pH caused by the introduction of HCl. It should be noted that this reversible fluorescent characteristic may potentially be useful in applications involving reusable biosensors.

It has been reported that PDAs selectively form an inclusion complex with α-CD, but not with γ-CD[5-7]. Figure 4 shows the fluorescence spectra of the PCDA/BO558 vesicles after reaction with cyclodextrins (α-CD and γ-CD) at different concentrations. All spectra were measured after reaction for 20 min because the color transition achieved a maximum after approximately 20 min[5]. Significant increases in fluorescence intensity were observed upon exposure to α-CD. However, exposure to γ-CD (Figure 4(B)) resulted in negligible changes when compared to that observed in α-CD (Figure 4(A)). These results indicate that conjugated polymer vesicles composed of PCDA and BO558 can selectively detect α-CD over γ-CD. In addition, the higher the concentration of α-CD is, the stronger is the fluorescence intensity. Figure 5 shows the net fluorescence increase acquired from the fluorescent spectroscopic measurements, which were compared to our previous detection results using colorimetric responses with concentration[5]. Selective detection by heterogeneous PCDA/BO558 vesicles of α-CD was clearly demonstrated at various concentrations. It would be worthwhile to compare this net fluorescence increase with the colorimetric response in view of sensing performance. It is suggested that the conjugated polymer vesicle sensors employing the FRET phenomena are valid for a wider range of concentrations than the pure color-changing PDA vesicle sensors that show saturated detection performance at higher concentrations.

Conclusions

We demonstrated that PDA/BO558 vesicles composed of PCDA and BO558 selectively detected the inclusion complexation of α-CD using fluorescent spectroscopy. Selective detection by heterogeneous PCDA/BO558 vesicles of α-CD over γ-CD was clearly demonstrated at various concentrations. When compared to the colorimetric response, the fluorescence changes of the conjugated polymer vesicle sensors employing FRET phenomena were valid for a wider range of target concentration. These FRET characteristics of PCDA/BO558 vesicle sensors would apply to a variety of biomolecular recognition events in the future.

Materials and Methods

Materials

10, 12-Pentacosadiynoic acid (PCDA: 99.9%), purchased from GFS Chemicals Inc. (USA), was used without further purification. 4,4-Difluoro-5-(2-thienyl)-4-bora-3a,4a-diaza-s-inda-cene-3-dodecanoic acid (BO558) was purchased from Molecular Probes (USA). Deionized water (with an initial resistivity of 18 MΩ · cm) and chloroform ($CHCl_3$: 99+%, Fluka) were used as solvents. Cyclodextrins, NaOH, and HCl were purchased from Aldrich and used as received.

Preparation of Polymerized PCDA/BO558 Vesicles

10, 12-Pentacosadiynoic acid and BO558 (molar ratio=100 : 0.25) were dissolved in chloroform in a test tube and the solvent was removed by purging with nitrogen gas stream to give thin films of the lipid on the tube's glass surface. 30 mL of deionized water was then added to yield a total 1 mM lipid concentration. The sample was heated at 80°C for 15 min and sonicated for 15 min with a probe-tip sonicator at 25% power (Fisher Scientific, USA). The sample was then filtered through a filter with 0.8-μm pores. The filtrate was collected and cooled at 4°C for 12 h. Polymerization was carried out by exposing the sample to the UV light of 254-nm wavelength at the intensity of 1 mW/cm^2 to give blue-colored vesicles.

Fluorescent Spectroscopy

Fluorescence changes were measured on a Hitachi F7000 fluorescence spectrophotometer (Japan) with an excitation wavelength of 495 nm. The emission spectra were obtained in the range of 520-700 nm. 2 mL of sample solution was used for the temperature effect-experiment. The sample solution was maintained for 10 min to a given temperature. A small quantity (20-60 μL) of NaOH or HCl solution was added to 2 mL of vesicle solution for the pH effect-experiment. Then, 2 mL of mixed solution (volume ratio of vesicle solution and CD solution is 1 : 1) was measured to confirm the application as chemical sensors. To obtain the spectra after saturation, fluorescence spectra were collected following 20 min of reaction of mixed solution.

Colorimetric Response (CR)

The CR values were calculated from the well-defined equation, $CR=(PB_0-PB_f)/PB_0\times 100\%^2$, where PB_0 and PB_f are percent blue before and after the color transition ($PB=A_{640nm}/[A_{640nm}+A_{550nm}]$), respectively.

Acknowledgements

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