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Enhanced Sensitivity for Hydrogen Peroxide Detection: Polydiacetylene Vesicles with Phenylboronic Acid Head Group

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Abstract It was recently reported that, besides UV irradiated polymerization, polymerization of diacetylene compounds could also been initiated by radicals generated from enzyme catalyzed hydrogen peroxide (H₂O₂) decomposition. A new optical sensing method for H₂O₂ was proposed based on this phenomenon. However, the sensitivity of this method is relatively lower than existed ones. In the present work, phenylboronic acid (PBA) functionalized 10, 12-pentacosadiynoic acid (PDA-PBA) was synthesized and its vesicles were formed successfully as colorimetric sensor for H₂O₂ detection. It was found that color change during the polymerization of vesicles composed of the PBA modified monomer is much stronger than that of the non-modified one. The response of PDA-PBA vesicles to H₂O₂ is 16 times more sensitive than that of the PDA. The absorption of PDA-PBA at 650 nm is linearly related to the concentration of H_2O_2 and a detection limit of $\sim 5 \mu$ M could be achieved.

Keywords Polydiacetylene · Hydrogen peroxide · Vesicles · Modification · Phenylboronic acid · UV light

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Introduction

Hydrogen peroxide (H_2O_2) has been widely used in bleaching, cleaning and disinfection [1]. It is also a by-product of oxidative metabolism [2]. The detection of H_2O_2 is of practical importance in food, clinical, modern medicine, environmental monitoring and various other industries [3, 4]. Many methods such as chemiluminescence [5, 6], electrochemical methods [7–9] and chromatography [10] have been developed for the detection of H_2O_2 . However, these detection methods need either expensive instruments or reagents. Therefore, optical biosensors are of the significant interest for the detection of H_2O_2 due to their low cost and simple instruments [11].

Recently, conjugated polymer colorimetric biosensor has become popular due to its high sensitivity, selectivity and practicality [12–16]. We previously reported a free radical triggered polymerization of 10, 12-pentacosadiynoic acid (PCDA) vesicles as a colorimetric reagent for H₂O₂ detection [17]. The polymerization of well-packed diacetylene monomers can be induced by the hydroxyl radical produced from the horseradish peroxidase (HRP) catalyzed H₂O₂ decomposition reaction [18–20]. The color of the reaction mixture changes from colorless to blue during the polymerization. Under the optimal conditions, the colorimetric detection of H₂O₂ can be completed with 20 µg/mL HRP (pH = 7.0) at 30 °C in 30 min. However, the previously reported PCDA-based method showed a relatively higher detection limit of ca. 80 µM which has no advantage compared to other methods [17].

The topotactic polymerization of diacetylene is generally known to be acutely sensitive to the molecular order of molecular assemblies. So, the molecular assemblie of the diacetylene determines not only the ability to form stable vesicles but also its chromic behavior. Besides the total chain length and position of the diacetylenic unit within the chain,

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chemical nature of the headgroup is a key structural factor for diacetylene compounds. Interactions between neighboring diacetylene molecules via their headgroups are especially important for signal amplification in sensors and this could be tailored by synthesis [21]. Niwa et al. prepared LB film of 10, 12-pentacosadiynamide phenylboronic acid (DA-PBA) and got a blue polymer film upon UV irradiation [22]. It is found that dehydration of boronic acid groups constrained in position on the surface of a two dimensional monolayer assembly would form cross-linked boronic anhydrides. Lee et al. also successfully prepared phenylboronic acids functionialized diacetylene network films on solid substrates [23]. In the present work, a polydiacetylene vesicle bearing phenylboronic acid group was prepared for the first time and used as a more sensitive colorimetric biosensor for H_2O_2 detection.

Synthetic Routes

Purification of PCDA

The commercial PCDA was purified for further use. Briefly, PCDA solid powder was dissolved in methylene chloride and filtered through 0.45 μ m membrane to remove the poly-PCDA. The methylene chloride was evaporated with a rotary evaporator and the resultant PCDA monomer was stored at -20 °C in the dark.

Synthesis of APCDA

Figure 1 shows the synthesis route for the mediate product 10, 12-pentacosadiynoic acid 2-aminoacetamide (APCDA). Briefly, 2.45 mmol purified PCDA monomer in 20 mL tetrahydrofuran was mixed with 2.4 mmol N-hydroxysuccinimide and 2.45 mmol 1-ethyl-3(3-dimethyl ammonium propyl)-carbodiimide. The mixed solution was allowed to react for



Fig. 1 The synthesis route of APCDA

24 h under 350 rpm mechanical stirring in the dark. The reaction solution was then evaporated to dryness with the rotary evaporator. The dried residue was re-dissolved in 30 mL chloroform and extracted with 20 mL saturated sodium chloride solution 6 times. The organic phase was collected and dried over anhydrous magnesium sulfate for 12 h. The product was dissolved in 50-70 mL chloroform. Ten milliliters ethylenediamine (4 mmol in dichloromethane) was added to the product solution dropwise. The pH of the solution was adjusted to 9.0 with triethylamine. The reaction was allowed to precede under 300 rpm mechanical at 30 °C in the dark. The reaction solution was then evaporated to dryness and the product, APCDA, was purified on a silica gel column with an eluant of chloroform: methyl alcohol (5:1). The yield of APCDA is 83-94 %. Figure 2 is the ¹HNMR of APCDA and data are as follows: ¹HNMR (300 MHz, CDC13): δ (ppm) 0.78–0.81(t, J = 9, 3 H), 1.11–1.85 (m, 36 H), 2.14–2.16 (t, J = 6, 3 H), 2.24–2.26 (t, J = 6, 2 H), 3.31–3.34(dd, J = 9, 2 H), 7.19(brs, 1 H).

Synthesis of APCDA-PBA

The synthesis route of APCDA-PBA is present in Fig.3. Ten grams of 4-carboxyphenylboronic acid (PBA, 60.3 mmol) were added into a two-necked flask. Thionyl chloride (150 ml) was then added to the flask in a N_2 atmosphere. The mixture was magnetically stirred (350 rpm) for 24 h at 88 °C. The reaction mixture was then evaporated to dryness and the dry residue was re-dissolved in 60 ml anhydrous tetrahydrofuran. Chloroform (20 ml) was added dropwise to 3 mmol of APCDA in a N₂ atmosphere with an ice-bath. A drop of dimethyl formamide (DMF) was added after 30 min and kept in the ice-bath for another 2 h in the dark. After that, the obtained mixture was added dropwise to the previous prepared PBA solution and allowed to react for 20 h in the dark. The reaction mixture was evaporated to dryness with the rotary evaporator and the product was purified on a silica gel column with an eluant of chloroform: methyl alcohol (7:1). The yield of APCDA-PBA is between 91 and 94 %. Figure 4 is the ¹HNMR of APCDA-PBA and data are as follows: ¹HNMR (300 MHz, CDCl3): δ (ppm) 0.78–0.81(t, J = 9, 3 H), 1.18–1.58(m, 36 H), 2.14–2.17(t, J = 9, 4 H), 2.35– 2.37(t, J = 6, 2 H), 2.87(s, 1 H), 2.94(s, 1 H), 7.05-7.06 (d, J = 3, 2 H), 7.19(brs, 2 H), 7.84–7.86 (d, J = 6, 2 H)..

Preparation of APCDA-PBA Vesicle

The produced APCDA-PBA (14.98 mg) was dissolved in 4 ml DMSO and 36 ml distilled water, heated to 70 °C and sonicated for 15 min. The solution was filtered and stored at 4 °C in the dark overnight.

Fig. 2 ¹HNMR of APCDA



Results and Discussion

Characterization of APCDA-PBA

The reaction substrate (PCDA), mediate product (APCDA) and product (APCDA-PBA) were confirmed with FTIR (Fig.5). The asymmetric stretching vibrations of CH₂ groups of the diacetylene side chains can be found at 2928 cm⁻¹ and 2852 cm⁻¹. The peak at 2349 cm⁻¹ can be ascribed to the vibrations of the unsaturated double bond CH = CH in the diacetylene monomer. These feature FTIR peaks are shared by all three different diacetylene derivatives. APCDA showed an absorption peak at 3324 cm⁻¹, which was assigned to the stretching vibrations of N-H bond in amide. The absorption peaks at 1697 cm⁻¹ and 1632 cm⁻¹ can be assigned to the stretching vibrations of hydrogen-bonded carbonyl in carboxy bond and the stretching vibrations of hydrogen-bonded carbonyl in amide, respectively. The new peak at 1070 cm⁻¹ is ascribed to the C-N bond in amide. These results indicate that

the ethylene diamine monomer was successfully coupled with the PCDA. APCDA-PBA showed three new absorption peaks at 1810 cm⁻¹, 860 cm⁻¹ and 1551 cm⁻¹, which were assigned to the stretching vibrations of C = C and stretching vibrations of C-H and B-O, respectively. These indicate that phenylboronic acid was coupled with the APCDA successfully.

The microstructures of poly-PCDA and poly-APCDA-PBA vesicles were imaged with a SEM (Fig.6). The poly-PCDA vesicles are composed of large spherical particles, while poly-APCDA-PBA vesicles composite of smaller rodshaped and square particles. This can be explained that amphiphilic monomers with achiral headgroups usually form spherical liposomes and chiral amphiphiles form nonspherical structures such as helices and tubules [24]. Similar small particles can also be found in polydiacetylene-amino/1, 2-dimyristoyl-SN-glycero-3 -phosphocholine (PDA/DMPC) mixed vesicles, which can improve the UV-Vis absorption of the vesicles and enhance the color change [25]. The PDA/



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Fig. 4 ¹HNMR of APCDA-PBA



DMPC mixed vesicle coupled with antigen has a flat shape, which can be attributed to its inherent variability and instability in preparation process [26]. Morphology change of vesicles can usually affect their detection sensitivities and stabilities [27–29]. The smaller vesicles provide more surface binding sites and thus improve the detection sensitivity of the colorimetric method [30].

UV Light Trigged Polymerization

The polymerization of diacetylene vesicles is initiated by UV light. The effects of irradiation time on the polymerization process of PCDA and APCDA-PBA were investigated. The absorption of the poly-PCDA at 650 nm (A_{650}) increased from 0.06 to 0.21 when the irradiation time was increased from 1 min to 2 min (Fig.7a). Meanwhile, A_{650} of poly-APCDA-PBA reached as high as 0.65 with 1 min irradiation. When irradiation time was increased to 2 min, the blue phase of poly-APCDA-PBA shifted to the purple phase and an absorption



Fig. 5 FTIR spectra of PCDA (*red line*), APCDA (*blue line*) and APCDA-PBA (*green line*)

peak appeared at 550 nm. A_{650} did not show significantly change. Thus, 1 min UV irradiation time is enough for the polymerization of APCDA-PBA. These also indicate that the polymerization of APCDA-PBA is easier to be trigged



Fig. 6 SEM images of poly-PCDA vesicles (**a**) and poly-APCDA-PBA vesicles (**b**) The polymerization was initiated with 254 nm UV light at a distance of 30 cm



than that of PCDA alone. The much higher absorption of the poly-APCDA-PBA in blue region (Fig.7b) indicates the high sensitive of colorimetric method with APCDA-PBA vesicle.

Detection of H₂O₂ with APCDA-PBA Vesicle

The potential application of APCDA-PBA vesicles in the quantification of H_2O_2 was explored. Figure 8a shows the UV-Vis absorption spectra of APCDA-PBA vesicles polymerized with various concentration of H_2O_2 ranging from 0 to 100 mM. It is clear the peak absorption at 650 nm increased with the increase of H_2O_2 concentration. APCDA-PBA vesicles can respond to as low as 5 μ M H_2O_2 , which is 16 times more sensitive than PCDA vesicles. In addition, the absorption of APCDA-PBA vesicles at 650 nm is significantly higher than that of PCDA vesicles [17] under same conditions, which

is consistent with the results of its UV-induced polymerization reaction (Fig.7b). Figure 8c shows the images of APCDA-PBA vesicles being polymerized with the different concentration of H_2O_2 from 5 μ M to 100 mM. It is clear that the intensity of the blue color is significantly increased with the increase of the concentration of H_2O_2 and the color change of APCDA-PBA vesicles is more dramatic than that of PCDA vesicles [17] under same conditions.

The possibility of the quantification of H_2O_2 with APCDA-PBA vesicles was explored. The relationship between the absorption of poly-APCDA-PBA at 650 nm and the concentration of H_2O_2 is fitted in a linear regression logA = -0.719+0.187log*c* (R² = 0.991, *n* = 7) (Fig.8b), where A is the absorbance of poly-APCDA-PBA at 650 nm and c is the concentration of H_2O_2 in μ M. The lowest detectable concentration of H_2O_2 is ~5 μ M.

Fig. 8 a UV-Vis absorption spectra of the APCDA-PBA vesicles polymerized with various concentrations of H₂O₂. b The linear calibration curve of the absorption of APCDA-PBA at 650 nm to the concentration of H₂O₂. Error bars represent the standard deviation for three indeppent measurements. c Typical images of the APCDA-PBA vesicles polymerized with different concentrations of H₂O₂ in the presence of 20 µg/mL HRP. The polymerization was conduct at pH = 7.0 and 30 °C for 30 min



Conclusions

In summary, the response of polydiacetylene vesicles to H_2O_2 can be effectively enhanced by the introduction of phenylboronic acid group. The absorption of PDA-PBA vesicles at 650 nm and the concentration of H_2O_2 are linearly related. The detection limit of the PDA-PBA vesicle based colorimetric method is ~5 μ M H_2O_2 , which is 16 times lower than that of the PDA vesicles based colorimetric method. The PDA-PBA vesicle is a promising tool for the detection of H_2O_2 in modern medicine, environmental and biotechnology monitoring.

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