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Article

Chromatic Detection of Cholesterol Using Polydiacetylene Vesicles

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Abstract

In this study, a new system to determine the concentration of cholesterol using a color change was developed. The system comprised diacetylene vesicles and cholesterol oxidase (ChOx). 10,12-Pentacosadiynoic acid (PCDA) was used as the diacetylene compound, and PCDA vesicles were formed using sonication. The H_2O_2 produced during the reaction between cholesterol and ChOx was used to initiate the polymerization of the PCDA in the vesicles. During polymerization, the vesicles changed from colorless to blue. Therefore, the cholesterol concentration was proportional to the intensity of the blue color. The absorption at 665 nm indicated that the blue color was directly proportional to the cholesterol concentration. This indicates that the system can be used for cholesterol detection. The minimum cholesterol concentration detected using this system was 1.0 mM.

Keywords: *Cholesterol detection, Color change, Polydiacetylene, Cholesterol oxidase*

1. Introduction

Cholesterol is an important component in the human body and is essential for the synthesis of hormones, vitamin D, and cell membranes. Therefore, approximately 200 mg/dL of cholesterol is present in the blood of healthy adults. However, cholesterol is the main cause of arteriosclerosis, and many people die from this cardiovascular disease. Consequently, the amount of cholesterol in the blood must be controlled to maintain a healthy state. The blood cholesterol concentration is an important numerical value in medical examinations[1].

Various sensors that can measure cholesterol concentration have been developed. These sensors can be classified into two types. One type uses enzymes[2-8] such as cholesterol oxidase (ChOx) and cholesterol esterase, whereas the other type does not use enzymes[9-13]. Enzyme-based sensors have superior sensitivity and selectivity compared to sensors without enzymes; however, they are more expensive [14]. Enzyme-free sensors can be produced using various methods. Molecular imprinting technique can be used to enzyme-free sensor. Although the selectivity and activity of these sensors are lower than those of other sensors, they can be manufactured using cheaper and easier methods. Consequently, these sensors have attracted significant attention[15]. Typically, three-dimensional crosslinked polymeric materials are used to fabricate sensors using molecular imprinting methods. However, the author introduced that cholesterol sensors using molecular imprinting methods were formed on a two-dimensional surface which was formed with the self-assembled monolayer (SAM) on the gold plate using the thiol compound, or on a thin membrane which was formed with the very thin poly(methyl methacrylate) coating on SAM[16-18]. Although ChOx was used in this study, the detecting method used in this study was the color measurement which is cheaper and easier methods.

Polydiacetylene (PDA) is a polymeric material containing alternately conjugated double and triple bonds. Because the color of PDA can be changed from blue to red by external stimuli, it has been used as a color-changing material for sensors[19,20]. The author has previously developed several sensors using PDA[21-25]. The most frequently used commercial raw material for the synthesis of PDA is 10,12-pentacosadiynoic acid (PCDA). When sonicated in water, it forms vesicular structures. Under UV irradiation the diacetylene groups in the PCDA vesicles polymerize to form PDA. The vesicle solution changes from colorless to blue during photopolymerization. The blue-to-red color change of PDA vesicle solutions under external stimuli has often been exploited to fabricate sensors; however, the author was the first to utilize the polymerization step, in which the color changes from colorless to blue, in the sensing mechanism[26]. In the previous study, the target material was glucose, and the results showed that the glucose concentration was directly proportional to the intensity of the blue color.

In this study, the H_2O_2 that forms during the reaction between cholesterol and ChOx was used as the initiator to polymerize PCDA vesicles. The color change from colorless to blue during the polymerization of the PCDA vesicles was used to determine the cholesterol concentration.

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2. Experimental

2.1. Materials and Instruments

PCDA, ChOx, cholesterol, and Triton X-100 were purchased from Sigma-Aldrich. Vesicles were formed using a VC-505 ultrasonic processor (Newtown, CT, USA). The ultraviolet (UV) spectra were obtained using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). Transmission electron microscopy (TEM) image was obtained using a TEM (Zeiss LEO 912 Omega).

2.2. Formation of PCDA vesicles

PCDA (75.0 mg, 2.00×10^{-4} mol) was dissolved in 20 mL of chloroform in a 250 mL round bottom flask. The chloroform was evaporated under vacuum, and the PCDA remained as a thin membrane at the bottom of the flask. After the complete removal of chloroform, 100 mL of water was added to the flask. The mixture was sonicated at 70 °C for 30 min to form a 2.00 mM PCDA vesicle solution.

2.3. Detection of cholesterol

The analyses were conducted under various conditions of ChOx concentration, cholesterol concentration, and detection time. In a typical detection system, 50 μL of ChOx solution (2.0 mg/mL in phosphate buffer solution [PBS]) and 1.00 mL of a 2.00 mM PCDA vesicle solution were mixed, and 0.45 mL of 20.0 mM PBS (pH 7.00) was added to the solution. Then, 0.500 mL of cholesterol solution was added. Therefore, the final concentrations of PCDA, ChOx, and PBS were 1.00 mM, 75 μg/mL, and 5.0 mM respectively. The detection temperature was 30 °C. The cholesterol standard solution was prepared by dissolving 773 mg of cholesterol and 3.75 g of Triton X-100 in 25 mL of water at 70 °C.

3. Results and discussion

3.1. Formation of PCDA vesicle and cholesterol detection system

PCDA was used as the diacetylene compound in this study. This compound contains conjugated two acetylene functional groups on the long carbon chain and one carboxylic acid functional group at the end of the carbon chain. Therefore, PCDA contains both a long hydrophobic carbon chain and a hydrophilic carboxylic acid group. When PCDA is sonicated in water, a round vesicular structure is formed, as shown in Figure 1. The structure can be confirmed using TEM. The author previously reported the TEM results for PCDA[26]. The average diameter of the vesicles in this study was 270 ± 80 nm. The diacetylene groups in PCDA were arranged side-by-side in the vesicle structure. Therefore, these diacetylene groups can be easily polymerized. After the diacetylene groups were polymerized to PDA, the vesicle solution changed from colorless to blue. UV irradiation is a common method to initiate polymerization; however, in this study, H_2O_2 was used as the initiator, and the PCDA polymerization process was used to detect cholesterol. The chemical structure of PCDA, the vesicle structure obtained after sonication, the cholesterol oxidation reaction with ChOx, and the PCDA polymerization reaction with H_2O_2 are shown in Figure 1.

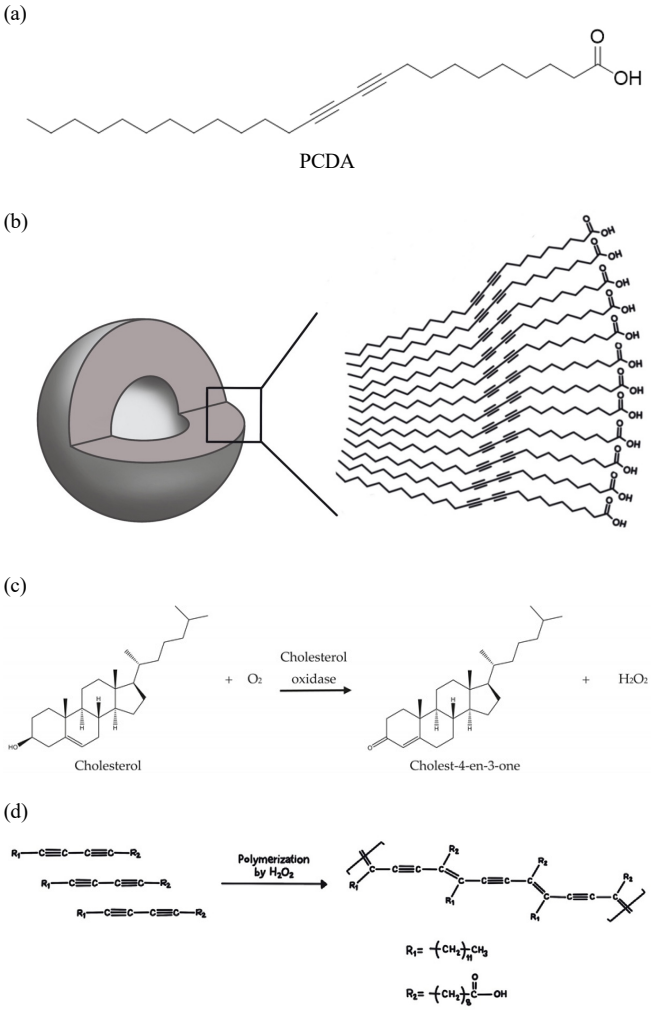

Figure 1. (a) Chemical structure of PCDA, (b) vesicle structure formed by PCDA, (c) reaction between cholesterol and ChOx, and (d) polymerization of PCDA by H_2O_2.

3.2. Effect of ChOx concentration on cholesterol detection

First, the effect of the ChOx concentration on the cholesterol detection activity of the system was determined. The concentrations of the other reagents were kept constant; the concentrations of PCDA, cholesterol, and PBS were 1.00 mM, 20.0 mM, and 5.0 mM respectively. The system was examined by increasing the ChOx concentration to 150 μg/mL. The detection temperature was maintained at 30 °C, and the results were recorded after 15 min. Photographs of the color change were captured from a top-down view of the UV cell. The UV spectra were recorded between 600 and 700 nm owing to the blue color of the solution. The changes in the intensity of the primary peak near 665 nm were recorded as a graph. The results are shown in Figure 2.

As shown in Figure 2, absorption increased proportionally up to a ChOx concentration of 100 μg/mL. At concentrations exceeding 100 μg/mL, the absorption value deviated from the proportional state, and

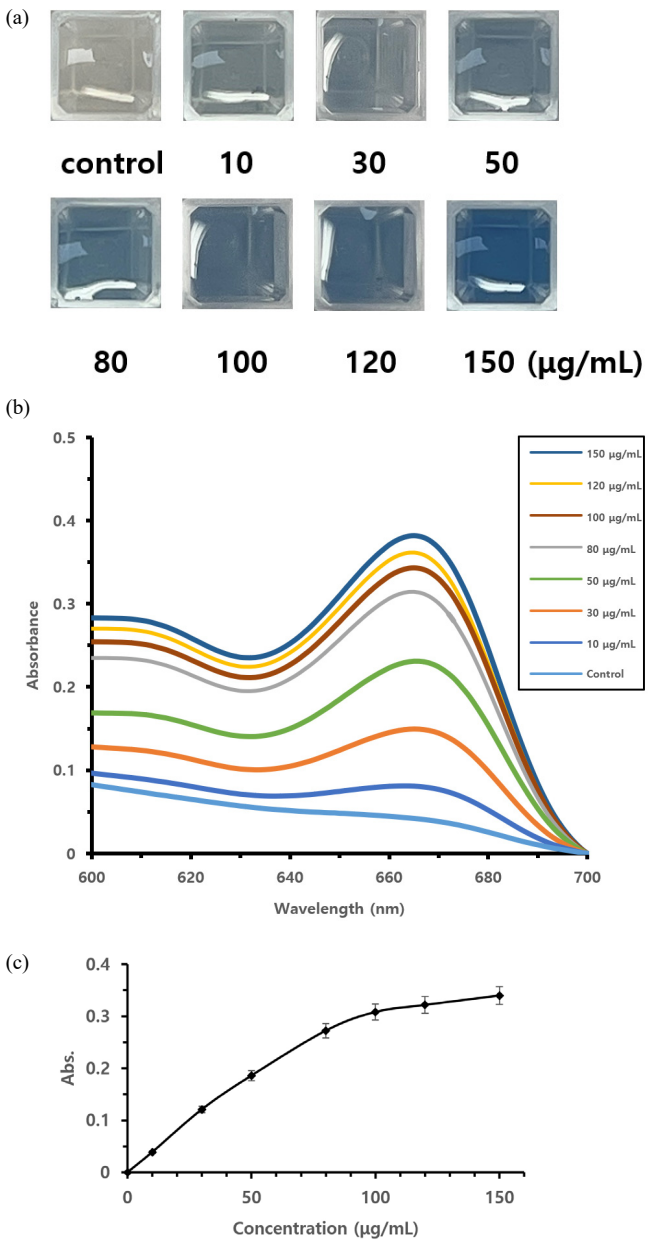

Figure 2. Effect of ChOx concentration on cholesterol detection using PCDA vesicle. (PCDA 1.00 mM, cholesterol 20.0 mM, PBS (pH 7.00) 5.0 mM, detection time 15 min, 30 °C). (a) Color change, (b) UV spectra, and (c) absorption change at 665 nm.

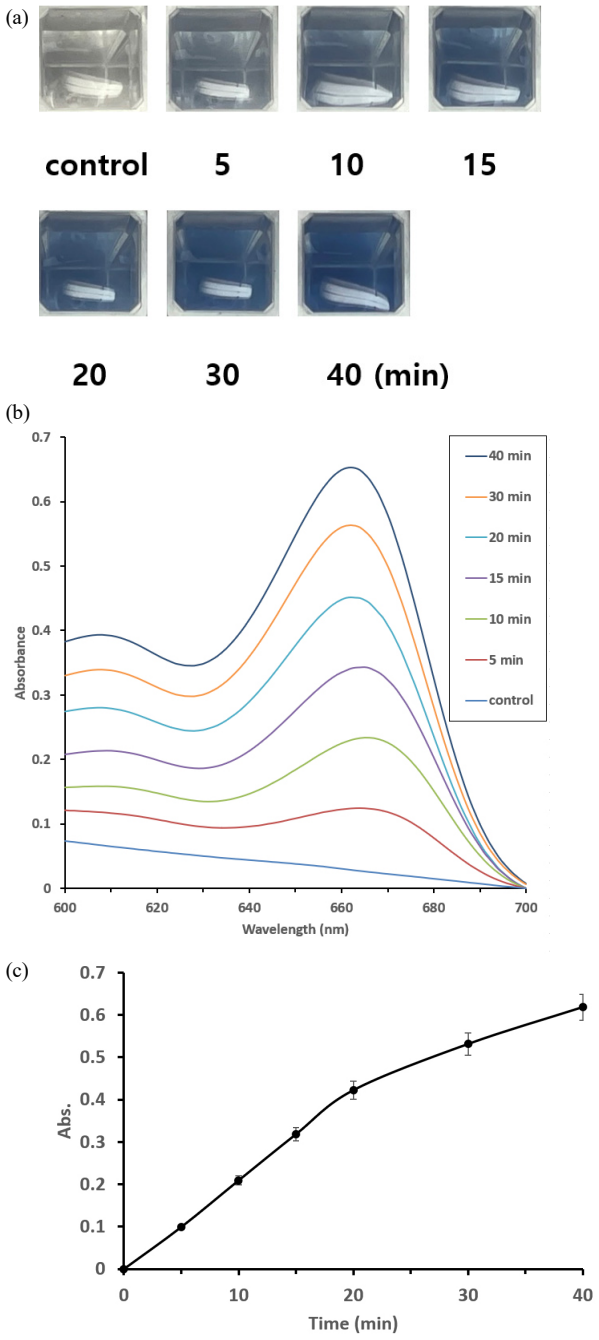

Figure 3. Effect of reaction time on cholesterol detection using PCDA vesicle. (PCDA 1.00 mM, ChOx 100 μg/mL, cholesterol 20.0 mM, PBS (pH 7.00) 5.0 mM, 30 °C). (a) Color change, (b) UV spectra, and (c) absorption change at 665 nm.

saturation phenomena were observed. Therefore, 100 μg/mL ChOx was selected as the standard concentration for this study.

3.3. Effect of detection time

In the next experiment, the effect of detection time was investigated. The concentrations of PCDA, ChOx, and PBS were 1.00 mM, 100 μg/mL, and 5.0 mM respectively. The cholesterol concentration was 20 mM, and the detection temperature was 30 °C. The results, a photograph of the color change, the UV spectrum between 600 and 700 nm, and the changes in the maximum absorption value near 665 nm are shown in Figure 3.

As shown in Figure 3, a blue color appeared after 5 min, and after 15 min, the blue color was clearly visible. Although the blue color was more intense at reaction times greater than 15 min, the intensity of the color at 15 min was sufficient to enable accurate results. Therefore, 15 min was selected as the standard detection time for this study.

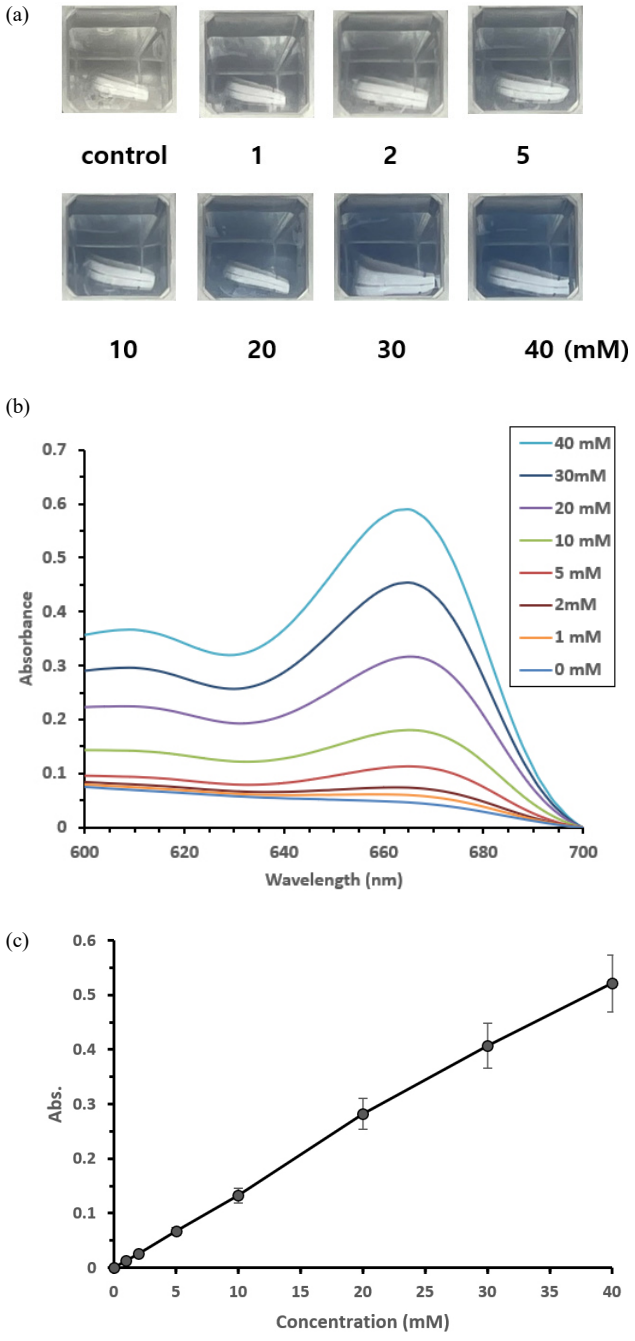

Figure 4. Effect of cholesterol concentration on chromatic detection using PCDA vesicle. (PCDA 1.00 mM, ChOx 100 μg/mL, PBS (pH 7.00) 5.0 mM, detection time 15 min, 30 °C). (a) Color change, (b) UV spectra, and (c) absorption change at 665 nm.

3.4. Effect of cholesterol concentration

Finally, the color change detection of the system based on the cholesterol concentration was determined. The concentrations of PCDA, ChOx, and PBS were 1.00 mM, 100 μg/mL, and 5.0 mM respectively. The detection time was 15 min. The examined cholesterol concentrations were 1, 2, 3, 5, 10, 20, 30, and 40 mM. The results, a photograph of the color change, the UV spectrum between 600 and 700 nm, and the change in the maximum absorption value near 665 nm are shown in Figure 4.

As shown in Figure 4, at a cholesterol concentration of 1.0 mM, a small color change was observed; however, this color change could still be detected visually. Therefore, 1.0 mM is considered as the limit of detection of this sensor system. The change in absorption near 665 nm increased proportionally with the cholesterol concentration. Therefore, this system can be used to detect cholesterol.

4. Conclusion

In this study, a system for detecting cholesterol concentrations based on a color change was developed. The system was constructed using PCDA vesicles and ChOx. The cholesterol reacted with the ChOx and H_2O_2 was produced. This H_2O_2 initiated the polymerization of the PCDA in the vesicles, and a blue color was observed after the polymerization. The optimal concentrations of PCDA, ChOx, and PBS were 1.00 mM, 100 μg/mL, and 5.0 mM respectively, and the optimal detection time was 15 min. The absorption increased proportionally with the cholesterol concentration. The minimum cholesterol concentration that could be detected using the system in this study was 1.0 mM.

Conflict of interest

The author has no relevant financial or non-financial interests to disclose.

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