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Polydiacetylene vesicles for hydrogen peroxide detection

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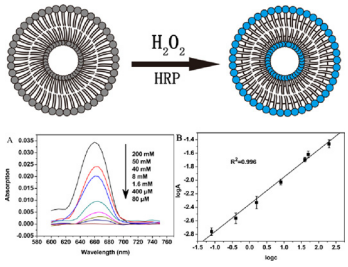
HIGHLIGHTS

- The PDA vesicles could be readily polymerized using free radical inductors.
- This study reported a novel method to detect H_2O_2 by using the PDA vesicles.
- The morphologies of PDA vesicles polymerized by UV and H_2O_2 are different.

GRAPHICAL ABSTRACT



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ABSTRACT

This communication presents a new approach for the polymerization of diacetylene vesicles by H_2O_2 in the presence of HRP (horse radish peroxidase). This offers a new optical sensing for H_2O_2.

Hydrogen peroxide (H_2O_2), previously known as a byproduct of aerobic respiration and a part of the phagocytic respiratory burst, has been recognized as one of the major risk factors in the progression of disease-related pathophysiological complications such as cancers and cardiovascular disorders [1,2]. In addition, H_2O_2 has wide industrial use in bleaching, cleaning, and disinfection, and its residue can harm the environment [3]. Furthermore, H_2O_2 is one of the products of almost all oxidase-mediated reactions [4]. Hence, the detection of H_2O_2 is of great importance for modern medicine, environmental monitoring, and various branches of industry. The detection of H_2O_2 has been previously achieved by measuring chemiluminescence, electrochemiluminescence, the luminescence of a metal ion, etc., which result from the excitation of the luminescent agents such as luminol or lanthanide ions via their reactions with H_2O_2 [4]. Among the many approaches that have been developed, optical biosensors possess an inherent advantage in that they do not require any complicated instrumentation or power supply, which makes them ideal for low resource settings. Recently, Shiang et al. reported a new luminescent assay using 11-mercaptoundecanoic acid-bound Au nanodots for the highly selective and sensitive detection of hydrogen peroxide [5]. The visual detection of H_2O_2 based on a H_2O_2 triggered sol–gel transition in combination with modified gold nanoparticles was also reported [6]. Recently, there has been significant interest in using

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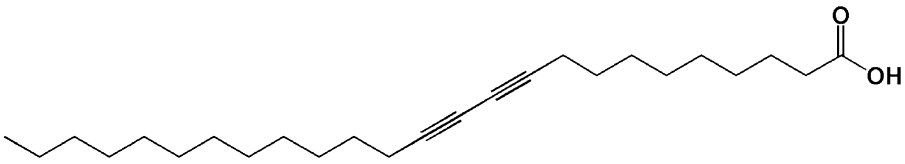

Scheme 1. Diacetylene monomer.

conjugated polymers as colorimetric biosensors for a variety of reasons (sensitivity, selectivity, and practicality) [7–10].

Polydiacetylene (PDA) is a unique conjugated polymer that shows a significant chromatic change in response to a variety of external stimuli such as temperature [11], pH [12], and particular molecules [13]. PDAs are normally prepared by UV (254 nm) or γ-ray induced polymerization of self-assembled diacetylene monomers (Scheme 1.) PDAs display blue color (ca., 640 nm maximum absorption wavelength), and the blue PDAs undergo a color shift to the red phase (ca., 540 nm maximum absorption wavelength) upon various environmental stimuli [14,15]. In addition, the red phase of PDAs is also weakly fluorescent. Most studies have focused on measuring the intensities of the colorimetric transitions (blue to red) and the fluorescence of PDAs for the detection of analytes [8]. In this work, we discovered that well-packed diacetylene monomers could also be readily polymerized using free radical inductors such as potassium persulfate (PPS), ammonium persulfate (APS), and H_2O_2. In view of the above discovery, here, we attempt to make use of the free radical triggered polymerization of diacetylene vesicles as a colorimetric method (transparent to blue) to detect H_2O_2. So far, there has been no report of using diacetylene vesicles to detect H_2O_2, and this work may open an avenue to utilize the polymerization of diacetylene vesicles for biosensing.

To demonstrate the feasibility of the method, a reaction mixture was prepared by mixing 500 μL, 1 mM diacetylene vesicles [16], and 500 μL PBS solution (100 mM, pH 7.0) containing appropriate amounts of PPS, APS, or H_2O_2 at a final concentration of 1 mM. After 12 h (reaction temperature controlled at 30 °C), different colorimetric responses occurred and the characteristic absorption bands at around 660 nm were observed in the resulting solutions that contained PPS or APS (Fig. 1). It can be noted that PPS induced stronger blue phase absorption than APS. Unfortunately, the addition of H_2O_2 resulted in no colorimetric response under the same condition. It is well known that HRP, a natural enzyme, has been used in H_2O_2 assay due to its sensitivity and selectivity in catalyzing the reduction of H_2O_2 in biologic solutions [17–19]. To improve the experiment, the solution of HRP (20 μL, 1 mg/mL, and 200–250 U/mg) was added to the reaction mixture, which gave a final H_2O_2 concentration of 200 mM and a final diacetylene concentration of 0.5 mM. The addition of HRP lead to an evident colorimetric reaction (Fig. 1) that gave the characteristic absorption at 660 nm, demonstrating that H_2O_2 can trigger the polymerization of diacetylene vesicles in the presence of HRP. Scanning electron microscopy (SEM) shows that the microstructures of the H_2O_2-induced polymerized vesicles are significantly different with the morphology of UV-induced polymerized vesicles (Fig. S1). Interestingly, the UV-induced polymerized vesicles form mostly spherical structures and to a lesser extent non-spherical structures such as tubules. In contrast, the H_2O_2-induced polymerized vesicles are in flat sheets rather than spheres or tubules and are larger than the UV-induced polymerized vesicles, as shown in the SEM images. Although the driving force for the formation of the flat sheet morphology is unclear, similar phenomenon has been observed in the poly (tetracosadiynoic acid)/1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) liposome system, whereas, the DMPC can increase the UV absorption intensity. Jelinek and co-workers previously presented the TEM image of PDA/DMPC particles, which appeared as sheets rather than vesicles [20]. Jiang and co-workers also showed that PDA/DMPC particles conjugated to antibody formed flat sheets [21]. Such observed variations in the self-assembled structures have been attributed to either the intrinsic variability in the liposome preparation method or the instability of the as-formed structures.

As shown in Fig. 2, the FTIR spectra of PDA vesicles polymerized by UV and H_2O_2 show peaks at 2846 and 2850 cm^{-1} due to the asymmetric and symmetric stretching vibrations of the CH_2 groups of the polydiacetylene side chains. The peaks at 1463 and 1693 cm^{-1} observed in both spectra can be assigned to the CH_2 scissoring vibrations and the hydrogen-bonded carbonyl C=O stretching vibrations, respectively.

To improve the detection limit and sensitivity, we further investigated the effects of HRP concentration, reaction time, reaction

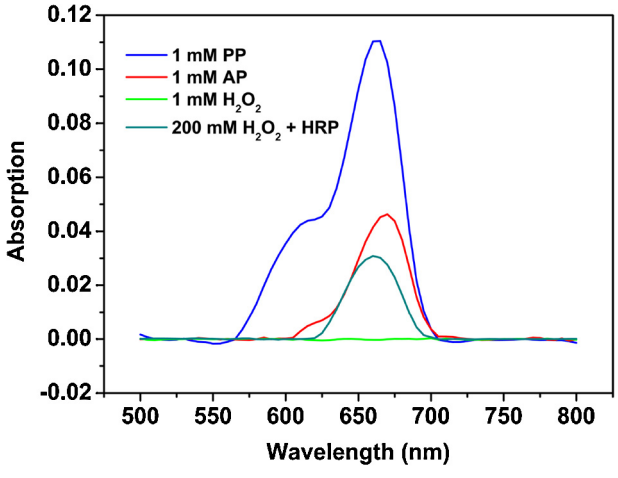

Fig. 1. UV/visible absorption spectra of the diacetylene vesicles in response to potassium persulfate (PP), ammonium persulfate (AP), H_2O_2, and H_2O_2 with HRP addition.

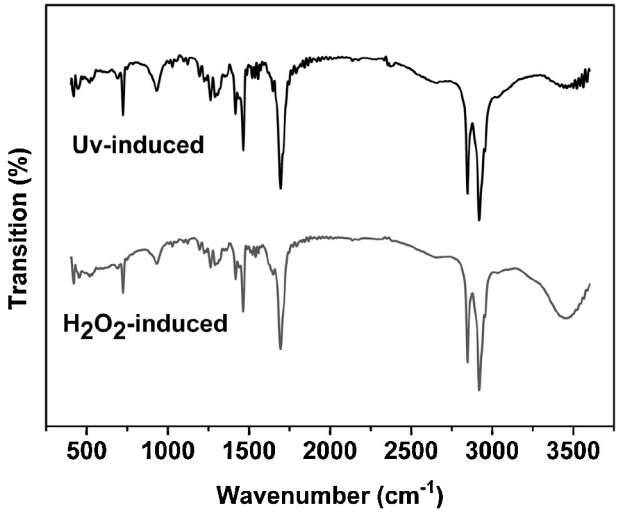

Fig. 2. FTIR spectra of polydiacetylene vesicle polymerized by UV and H_2O_2.