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Functionalized polydiacetylene-glycolipid vesicles interacted with *Escherichia coli* under the TiO_2 colloid

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Abstract

The interaction of the conjugated system between functionalized polydiacetylene-glycolipid vesicles and *Escherichia coli* was investigated under the bactericidal effect of TiO_2 colloid. With various pre-incubation and irradiation time, controllable bacteria quantity in the presence of TiO_2 colloid was obtained in real time. UV–visible and Raman spectra were utilized to monitor and evaluate the structural transition of bacteria-polydiacetylene-glycolipid vesicles conjugated system under the different conditions, which supplied the detailed information of the transform of the polydiacetylene backbone in real time. Thus controllable conjugated bio-interaction between two bio-interfaces was obtained through the introduction of the third factor and monitored in real time. This would be great aid in fundamental understanding more complex interfaces in the biological environment and would be applicable to the biomedical and biophysical fields.

Keywords: Polydiacetylene-glycolipid vesicles; *Escherichia coli*; TiO_2 colloid; Conjugated interaction

1. Introduction

Studies on the interactions between exotic macro-biomolecules and biomolecules on the cell surface, which play major roles in numerous biochemistry processes, are necessary to understand various biological processes. Most of bacteria and toxins use specific biomolecular recognition and binding at the cell surface as the first step toward invasion, such as special bio-interaction between mannose and *Escherichia coli* (*E. coli*) [1–4]. It has been reported that after assembled in order array, such as Langmuir–Blodgett (LB) films and vesicles, diacetylene can be polymerized into blue polydiacetylene (PDA) by ultraviolet light irradiation [5–8]. The external perturbations such as heat [9], and mechanical stress [10], could trigger the color transition of PDA from blue to red, where they are attributable to the changes in the effective conjugation length of the delocalized π-conjugated polymer backbone [11]. And when by coupling biomimicking molecular recognition with biomaterials of chromatic transition capability, polydiacetylene could also be triggered a chromatic phase transition, as biosensor for rapid colorimetric detection of special biological molecules, such as instant diction of toxins secreted by *E. coli* [8]. Hence a wide variety of diacetylenes have been studied in this way [12–17].

We previously showed that by organizing a synthesized glycolipid into polydiacetylene LB films, the interaction between glycolipid and *E. coli* could be detected by electrochemical method [18]. But the details on how these bacteria influenced the backbone of the PDA were limited. In this paper, we studied this influence through monitoring the structural transition by UV–visible and Raman spectroscopic technique. And TiO_2 colloid, an attractive photocatalyst for sterilization [19–23], was introduced into this bacteria–glycolipid–PDA conjugated system in order to obtain controllable quantity of *E. coli* in real time.

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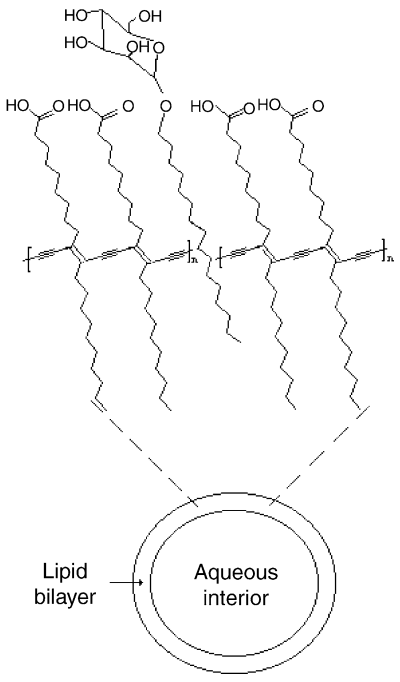
2. Experimental section

2.1. Materials

Mannoside derivative (Scheme 1, MC_{16}) was synthesized according to a procedure described elsewhere and confirmed by ^1H NMR. 10,12-Pentacosadiynoic acid (Farchan, Gainesville, FL) was re-crystallized with petroleum ether (30–60 °C). All these materials were dissolved in a chloroform solvent before use (1 mM).

2.2. Construction of the vesicles

To prepare polymerized vesicles, 5% MC_{16} and 95% 10,12-pentacosadiynoic acid were mixed in a conical flask, blown with N_2 for 90 min to remove chloroform completely, and a white thin film of the lipids on the bottom of the flask was yielded. Same amount of deionized water (resistivity of 18.3 MΩ cm^{-1}, Milli-Q, USA) was added (typically of 1 mM). Filled the sonicator bath with water, added two drops of detergent to the water, and heated the water to 72 °C, then placed the conical flask in the bath. The levels of water inside and outside the flask should be critically equal. After sonication for 30 min, the sample was kept warm for 2 h, cooled to the room temperature, and kept in dark at 4 °C for 48 h. Prior to polymerization, the vesicles were purged with N_2 for 20 min again after being warmed to the room temperature. The vesicles were polymerized using 254 nm light (1.8 mW/cm^2) for 285 s.



Scheme 1. Schematic representation of the mixed MC_{16}/PDA vesicles, indicating a part of the assembly of glycolipid and polydiacetylene.

2.3. E. coli K12 culture

E. coli K12 (44106-2) was used as model microorganism for colorimetric detection and bactericidal studies. *E. coli* K12 was cultured aerobically at 37 °C for 18 h on solid medium (pH 7.4) in 1 L broth containing 10% peptone, 5% NaCl and 20% agar. The solid medium was warmed-up and solved. The autoclave was operated at a pressure of approximate 15 lb/in.^{2} (at 121 °C) for 30 min. The bacteria on medium were washed with normal saline. Then the *E. coli* K12 was centrifuged at 1700 × *g* for 10 min, washed twice, and re-suspended in normal saline. The concentration of *E. coli* K12 was determined using a turbidimetry and was adjusted to the required final concentration (9×10^8 cells/ml).

2.4. Preparation of TiO_2 colloid

TiO_2 colloid solutions (4.55 mM) were prepared by hydrolysis of $TiCl_4$, which could be found elsewhere [24]. $TiCl_4$ (100 μl, 0.91 mmol) cooled to 0 °C was rapidly added to the 100 ml 0.2 M HCl solution under vigorous stirring in ice-water bath. Then after 2 h moderate stirring, 100 ml H_2O was added and stirred for another 30 min. The pH value of the TiO_2 colloid solution used in the experiment was adjusted from <3 to about 7.0 in order not to damage the normal growth of *E. coli* K12 and disturb the colorimetric of vesicles.

2.5. Controllable bacterial quantity

Diaphanous TiO_2 colloid solution and *E. coli* K12 suspension were mixed (volume ratio, 1:2) and pre-incubated for various time (0, 30, 60 min). 0.2 ml the mixture was piped into a Ø50 mm watch glass, irradiated (0.2 mW/cm^2) typically for 1 h, and then 1 ml vesicles were added, incubated for 2 min, compared with the sample without irradiation. The UV–visible absorption spectra of the sample were recorded by UV-360 photometer (Shimadzu, Japan). The other method for controllable bacterial quantity was yielded by various irradiation time. The mixture of TiO_2 colloid and *E. coli* K12 was pre-incubated for 60 min, and then the absorption spectra of the vesicles solution, which was incubated with the mixture of TiO_2 colloid and *E. coli* K12, were monitored as a function of irradiation time at wavelengths of 670 and 570 nm, and used to calculate the colorimetric response [8]. The light intensity at the suspension surface was measured to be 0.2 mW/cm^2.

2.6. Resonance Raman (RR) spectroscopy measurements

RR spectra were measured by using Microlaser Raman Spectrophotometer (made in France) at a resolution of 4 cm^{-1}. The 488 nm line with a power of 50 mW from an argon ion laser was used as excitation source. A polarizing beam splitter composed of two half-wave plates and a polarizing cube was used to control continuously the power of the

exciting radiation reaching the sample. The polarization of the 488 nm beam was oriented perpendicular to the entrance slit of the spectrometer in order to generate RR scattering. The vesicles were dropped into a watch glass (typically 0.2 ml). The laser beam was focused to a spot of 30 μm on the surface of the sample. To avoid the influence of the photoinduced thermochromism, the sample was placed in the conditions of lower temperature.

3. Results and discussion

3.1. UV–visible spectroscopy monitoring of the MC_{16}/PDA vesicles

Fig. 1 shows the UV–visible absorption spectra of the mixed MC_{16}/PDA vesicles under the various time of the UV irradiation. The vesicles became blue at once when irradiated with an UV light, as shown in Fig. 1 (5 s). Before 40 s, the main absorption peaks at 640 nm and a weak peak or the broad shoulder at ca. 600 nm were observed. The absorption intensity nearly saturated at around 285 s. Thus due to the difference of the polymerized extent with various irradiation time, the conjugating plane of PDA was changed gradually, and reached the saturated level.

In Fig. 2A, the UV–visible absorption spectra of blue vesicles before and after exposure to *E. coli* K12 are shown. Because the spectral changes were so distinct that the color change was visible with the naked eyes, particularly when the sample was placed against a white background (Fig. 2B). A quantitative value for the extent of blue-to-red color transition is given by colorimetric response (CR) [8], which is defined as: $CR(\%) = [(B_0 - B_v)/B_0] \times 100\%$, where $B = A_{blue}/(A_{blue} + A_{red})$, A is the absorbance at either the "blue" component in the UV–visible spectrum (670 nm) or the "red" component (570 nm), B_0 is the $A_{blue}/(A_{blue} + A_{red})$ ratio of the original vesicles, and B_v is the value obtained for the vesicle solution after incubation of *E. coli* K12. Here, CR was calculated as ca. 62%.

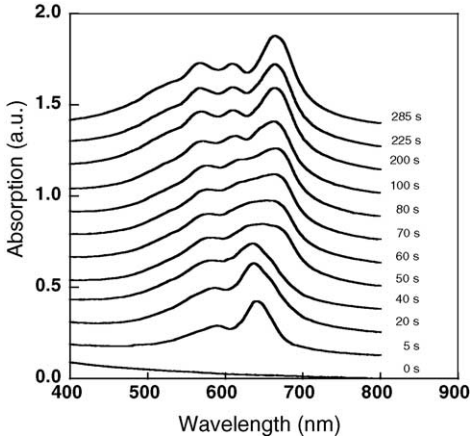


Fig. 1. UV–visible absorption spectral changes of the vesicles (1 mM) during polymerization induced by UV irradiation. The irradiation time is indicated at the right-hand side of each spectrum.

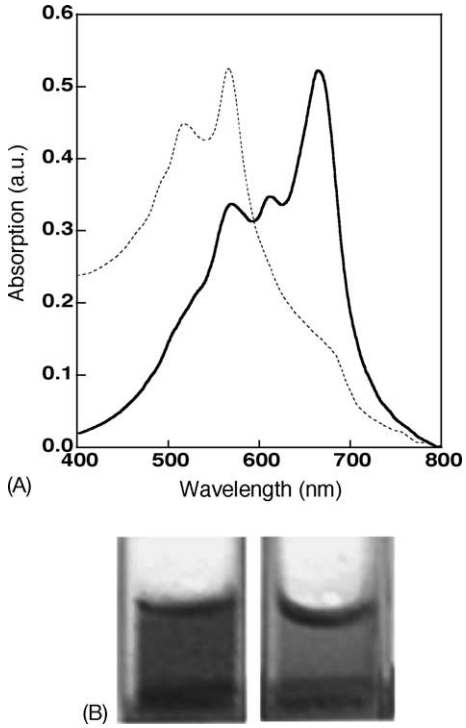


Fig. 2. Colorimetric transition of *E. coli* K12 using polymerized vesicles containing mannoside derivative. (A) Visible absorption spectra before (solid line) and after (dashed line, CR of 62%) incubation with *E. coli* K12. The solution was incubated with 9×10^8 cells/ml *E. coli* K12 for 2 min. (B) Photograph of vesicles before (blue) and after (red) incubated with *E. coli* K12.

CR of the vesicles as a function of *E. coli* K12 concentration was also measured, and the results were plotted in Fig. 3. Similar to the other results [25], sigmate behavior shown that

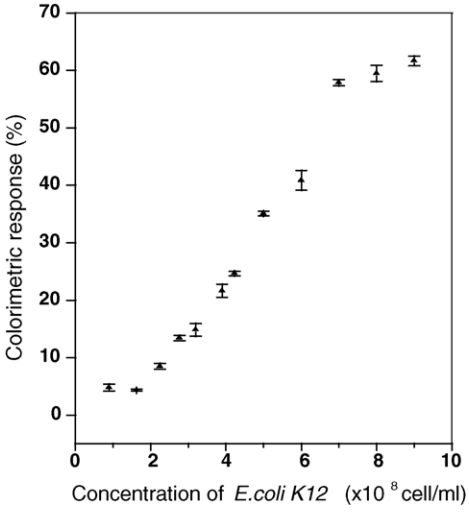


Fig. 3. Plot of the colorimetric response of mixed MC_{16}/PDA vesicles vs. *E. coli* K12 concentration. The vesicles were incubated with *E. coli* K12 for 2 min before the visible absorption spectrum was recorded. Each CR value was obtained in three independent experiments.

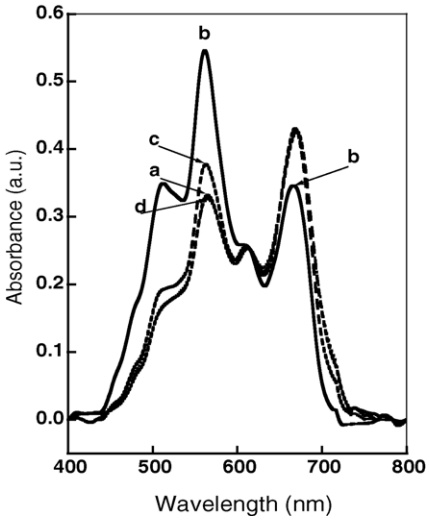


Fig. 4. UV–visible absorption spectra of MC_{16}/PDA vesicles irradiated for 1 h, with colloid TiO_2 (a), TiO_2 and *E. coli* K12 (1:2 in volume ratio) without pre-incubation (b), pre-incubated for 30 min (c), and 60 min (d). The volume ratio of TiO_2 or TiO_2–*E. coli* K12 suspension to the vesicles was 1:5 in final.

the colorimetric response to *E. coli* K12 leveled out at lower and higher concentration and increased in a linear fashion with the concentration of *E. coli* K12 in the range of 2×10^8 to 7×10^8 cells/ml.

3.2. Controllable bacterial quantity in the presence of TiO_2 colloid

Fig. 4 shows the UV–visible absorption spectra of mixed MC_{16}/PDA vesicles under the bactericidal effect of TiO_2 colloid in the different pre-incubation time. Without the pre-incubation of the mixture, the bactericidal effect of TiO_2 colloid was not observed and the vesicles remained red (Fig. 4b). With the increase of the pre-incubation time, the vesicles became blue gradually as the absorption intensity of vibronic band at 570 nm decreased while the main excitonic absorption at 670 nm increased (Fig. 4c and d). And when the pre-incubation time reached 60 min (Fig. 4d), the vesicles became blue again, which was similar to the vesicles incubated with TiO_2 colloid only (Fig. 4a). These results indicate that the different pre-incubation time can notably change the bactericidal effect of TiO_2, thus controllable quantity of *E. coli* could be obtained in this way. Considering the fact that the surface of TiO_2 is positively charged [26,27], and the surface of *E. coli* K12 is negatively charged, with proper more pre-incubation time, the TiO_2 colloid adsorbed the *E. coli* more tightly, and destructed more bacterial cells after irradiation of UV light [28].

The influence of the irradiation time on the colorimetric response of the suspension was also observed (Fig. 5). It seems that the destruction on the cell of bacteria by TiO_2 colloid is a gradual process. Within 30 min, the bactericidal effect of TiO_2 colloid was not obvious, and CR decreased less than 10%, which meant that most bacteria were survival. But if irradiated longer than 40 min, the CR was only 9.6%, meaning that most bacteria were killed. When irradiated for 60 min, the bacteria were killed completely, as the CR was very small (6.6%), almost the same as the value of the background (7%).

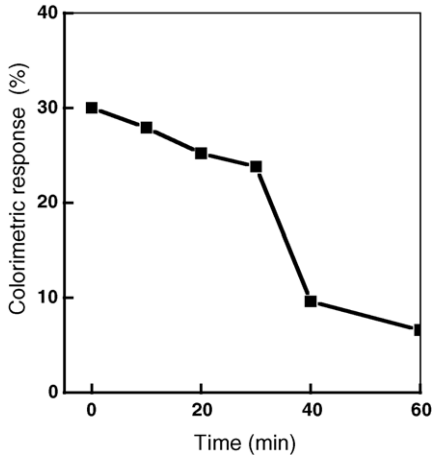


Fig. 5. Colorimetric transition of MC_{16}/PDA vesicles as a function of light irradiation time in the presence of TiO_2 in *E. coli* K12 suspension.

The effective bactericidal effect of TiO_2 colloid was in favor of obtaining controllable quantity of bacteria. In the presence of TiO_2 colloid with various time of the pre-incubation and irradiation, the quantity of bacteria in the system could be controlled in real time and at the same time the interaction of the bacteria–glycolipid–PDA conjugated system could be monitored by UV–visible spectra.

3.3. RR analysis of the transformation of the PDA backbone

RR analysis of MC_{16}/PDA vesicles recognized by *E. coli* K12 provided molecular information of this bio-interaction. The RR spectra of the original blue vesicles (Fig. 6a) consisted of two bands at 1446 and 2071 cm^{-1} which might be assigned, respectively, to the stretching modes of the double and triple bonds in the polymer backbone [9]. Moreover, there were two bands at 1511 and 2116 cm^{-1}, which might be assigned to the stretching modes of the double bonds in the polymer backbone. By contrast, when the vesicles directly interacted with *E. coli* K12, they changed from blue to red within 2 min (Fig. 6d), in which two major bands at 1511 and 2116 cm^{-1} might be assigned, but the bands at 1446 and 2071 cm^{-1} almost disappeared. When pre-incubated with a suspension of TiO_2 colloid and *E. coli* K12, and then irradiated, the bands at 1446 and 2071 cm^{-1} also appeared (Fig. 6c). Literatures [29–31] reported there existed two resonance structures in the polydiacetylene backbone (shown in Scheme 2), and the acetylene structure was energetically more favorable than the butatriene structure in the ground state of long chain polydiacetylene molecules. Therefore here the transformation between the double and the triple bonds might be attributable to a transformation between acetylene and butatriene. It was also noticed that the presence of TiO_2

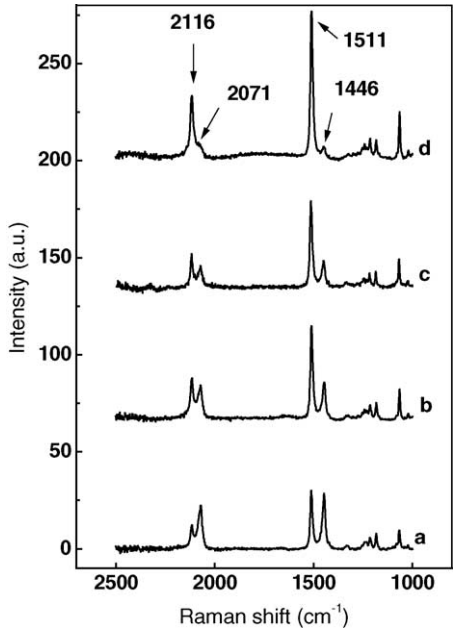
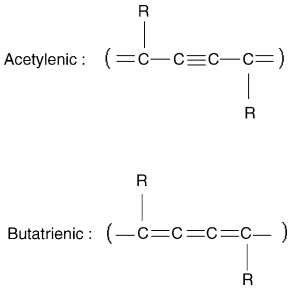


Fig. 6. RR spectra of MC_{16}/PDA vesicles irradiated for 1 h: (a) original vesicles; (b) with colloid TiO_2; (c) with *E. coli* K12 (6×10^8 cells/ml) and colloid TiO_2 suspension pre-incubated for 60 min; (d) with *E. coli* K12 (6×10^8 cells/ml).



Scheme 2. Schematic diagram of two resonance structures of the polydiacetylene.

colloid did not influence the double and the triple bonds of PDA (Fig. 6b).

From the results mentioned above, it is clear to demonstrate that (1) the TiO_2 colloid did not change the properties of mixed MC_{16}/PDA vesicles; (2) under the bactericidal effect of TiO_2 colloid, the quantity of the bacteria was controlled in real time and at the same time the transformation between acetylene and butatriene could be monitored by RR spectra.

4. Conclusion

Through the introduction of TiO_2 colloid to the conjugated bio-interaction between MC_{16}/PDA polydiacetylene vesicles and *E. coli* K12, controllable transformation between acetylene and butatriene of PDA was obtained and monitored in real time under the bactericidal effect of TiO_2 colloid. The combined utility of the UV–visible and RR spectra in real time provides us with more detailed information about the structural changes of PDA. The significance and uniqueness of this work is that controllable conjugated bio-interaction between two bio-interfaces was obtained through the introduction of the third factor and monitored in real time. This would be great aid in fundamental understanding more complex interface in the biological environment and would be applicable to the biomedical and biophysical fields.

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