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Enhanced affinochromism of polydiacetylene monolayer in response to bacteria by incorporating CdS nano-crystallites

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Abstract

By incorporating bio-specific receptors, such as *p*-10,12-pentacosadiyne-1-*N*-(3,6,9-trioxaundecylamide)-α-D-mannopyranoside (MPDA), into 10,12-pentacosadiyonic acid (PDA) monolayer, the MPDA/PDA monolayer underwent affinochromatic transition in response to the bacteria binding to the receptor. Here, we described a new method to study the membrane/macromolececular interaction between *Escherichia coli* (*E. coli*) and mannose and its relative affinochromism by modifying MPDA/PDA with CdS nano-crystallites (MPDA/PDA-CdS). CdS not only triggered the strong tropism of the bacteria but also reduced the rigidity of the MPDA/PDA backbone, resulting in the enhanced affinochromism. This discovery might be of significance in basic biophysical studies of membrane/macromolececular and designing novel biosensor.
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Keywords: Affinochromism; Polydiacetylene; CdS nano-crystallites; *Escherichia coli*

1. Introduction

Studies on the interactions between exotic macro-biomolecules and bio-membrane of the cell surface are necessary to understand various biological processes, such as special biointeraction between mannose and *Escherichia coli* (*E. coli*) [1,2]. It has been reported that diacetylene can be polymerized into blue polydiacetylene (PDA) by ultraviolet irradiation, and could be triggered an affinochromatic phase transition in response to the bacteria by incorporating bio-specific receptors [2–8]. In recent years, semiconductor nano-crystallites have attracted considerable attention and been used as powerful detection and encoding agents [9] due to their unique size-dependent chemical and optoelectronics properties [10]. And semiconducting nano-crsystallites (CdS, Ag₂S, FeS, for example) have already been easily prepared in a wide variety of different mediae such as taking LB technology to in situ generate nano-crystallites [11]. In this paper, we report our discovery that the affinochromism

of the PDA monolayer in response to *E. coli* was enhanced by the modifying the terminal carboxyl group of PDA with CdS nano-crystallites with the aid of LB technology.

2. Experimental

2.1. Chemicals

10,12-Pentacosadiyonic acid (PDA) was purchased from Farchan Laboratories (USA), and was re-crystallized from petroleum ether before use. *p*-10,12-Pentacosadlyne-1-*N*-3,6,9-trioxaundecylamide-α-D-mannopyranoside (MPDA) was provided by Dr. Peng Wang (Department of Chemistry, University of Miami). All other reagents used were of analytical grade without further purification.

2.2. Preparation of monolayer

Typically, 1.0 mM of 1:20 (mole ratio) MPDA/PDA which was dissolved in a mixed solution of 5:1 (v/v) chloroform and methanol was spread on the surface of KSV-5000 twin-compartment Langmuir trough [12]. The film was

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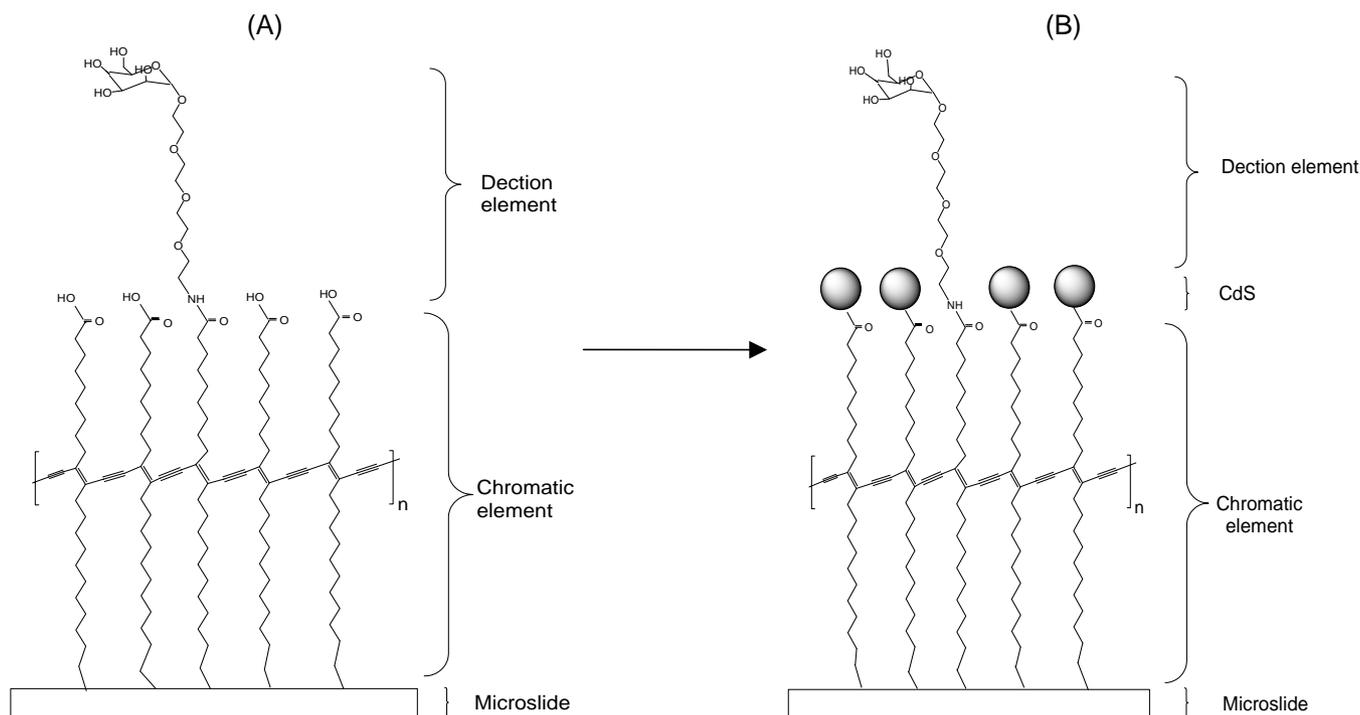
Scheme 1. Schematic representation of the construction of polymerized MPDA/PDA (A) and MPDA/PDA-CdS (B) monolayer.

compressed to a constant pressure (20 mN/m) at a speed of 4 mm/min, and allowed to equilibrate for 20 min. Then, it was irradiated with 254 nm light for 10 s (1.8 mW/cm^2), and transferred to the microslide's surface by horizontally lifting the microslide from the water surface.

2.3. CdS nano-crystallites modified PDA monolayer (MPDA/PDA-CdS)

The modification of the terminal carboxyl of PDA with CdS nano-crystallites (Scheme 1B) was achieved by in situ generation method [11,13]. Briefly, MPDA/PDA monolayer modified microslide was immersed in 10^{-4} M CdCl$_2$ solution for 2 h, and after that MPDA/PDA was exposed to H$_2$S gas for 1 h in a sealed glass bottle. The formation of CdS nano-crystallites on the headgroup of terminal carboxyl of PDA was confirmed by UV-Vis spectrum after taking out the background (Fig. 1). It can be seen that there was a typical absorption of CdS nano-crystallites at ca. 427 nm, and the size of CdS nano-crystallites was estimated to be ca. 3 nm [14]. The formation of CdS was also proved by XPS spectra, which was in agreement with the literature [15]. The binding energy values for S 2p and Cd 3d$_{5/2}$ were 161.8 and 405.5 eV, respectively (calibrated with C 1s, 248.6 eV).

2.4. Bacteria solution preparation

E. coli K12 (44106-2, obtained from School of Basic Medicine, Jilin University) was used as model microorgan-

ism for colorimetric detection and bactericidal studies, and was cultured aerobically at 37 °C for 18 h on solid medium (pH 7.4) in 1 L beaker containing 10% peptone, 5% NaCl, and 20% agar. The solid medium was warmed up and solved. The autoclave was operated at a pressure of ca. 15 lb/in.^{2} (at 121 °C) for 30 min. The bacteria on medium were washed with normal saline three times, centrifuged at $1700 \times g$ for 10 min, and at last diluted with the physiological saline to the concentration of ca. 9×10^8 cell/ml, which was determined by a turbidimetry.
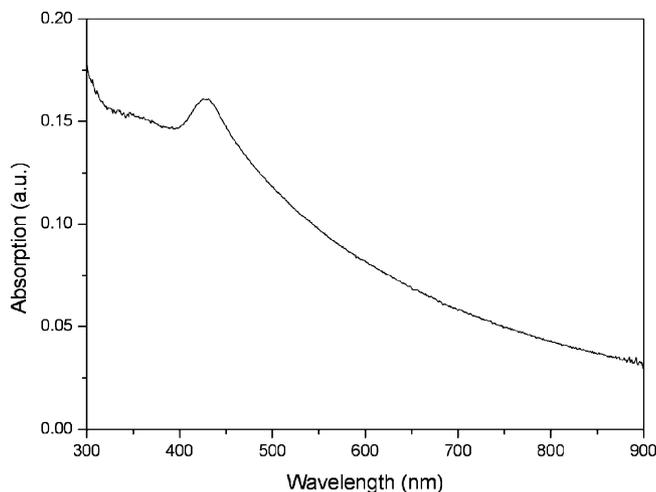

Fig. 1. UV-Vis spectrum of CdS nano-crystallites on the MPDA/PDA monolayer (after taking out the background).
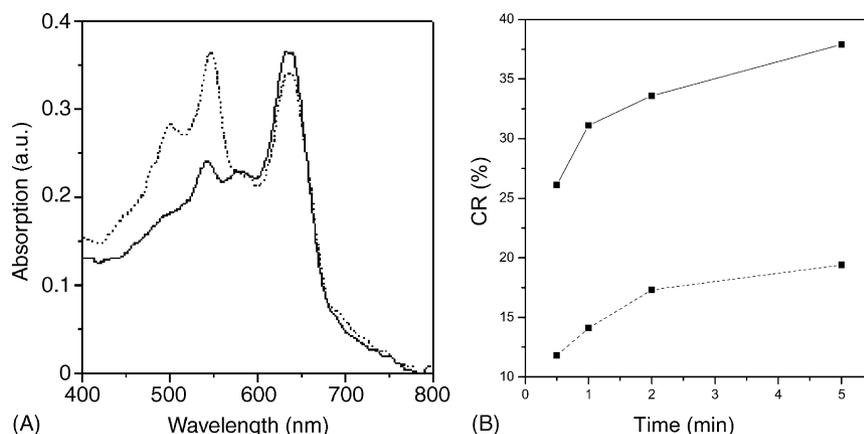
Fig. 2. (A) UV-Vis spectra of CdS nano-crystallites modified MPDA/PDA monolayer before (solid) and after (dash) incubation with *E. coli K12* bacteria for 30 s. (B) Colorimetric response (CR) of MPDA/PDA monolayer with (solid) and without (dash) CdS nano-crystallites modification under various incubation time with *E. coli K12* bacteria.

2.5. UV-Vis spectroscopy

UV-Vis spectra were measured by using UV-360 spectrometer (made in Japan, Shimadzu).

2.6. Resonance Raman spectroscopy

Resonance Raman spectra were measured on Raman infinity 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 samples. The polarization of the 488 nm beam was oriented perpendicular to the entrance slit of the spectrometer in order to generate RR scattering. To avoid the influence of the photo-induced thermochromism, the sample was placed in the condition of lower temperature.

3. Results and discussion

MPDA/PDA monolayer (Scheme 1A) had a typical maximum UV-Vis absorption at 640 nm, and a weaker absorption at 540 nm. When MPDA/PDA was incubated with *E. coli K12* [12] in 30 s, the color of the monolayer changed from blue to red. The maximum absorption at 640 nm decreased and the weaker absorption at 540 nm increased and the colorimetric response (CR) value[1] was ca. 11%.

After the monolayer was modified with CdS (Scheme 1B), the basic chromatic properties of PDA monolayer were almost not altered but with a much larger CR (Fig. 2A). The blue-colored monolayer also had a maximum absorption at 640 nm and a weaker absorption at 540 nm, while after incubation with *E. coli K12* for 30 s, the CR value increased to 26%. Under various time of incubation, this phenomenon was all observed (Fig. 2B).

The basic colorimetric properties of MPDA/PDA were not changed by the modification with CdS. It was confirmed by Resonance Raman spectra, which was demonstrated in Fig. 3. Both MPDA/PDA and MPDA/PDA-CdS monolayer had two typical major bands at 1507 and $2117 \, cm^{-1}$. These peaks mostly determined its colorimetric properties, and were assigned, respectively, to the stretching modes of the double and the triple carbon–carbon bonds in the blue MPDA/PDA backbone [16].

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[1] In order to quantify the response of a monolayer to a given amount of bacteria, the visible spectrum of the monolayer before to bacteria was analyzed as $B_0 = I_{640}/(I_{540} + I_{640})$. The same value (*B*) was calculated for monolayer exposed to *E. coli K12*. The colorimetric response of a monolayer is defined as the percent change in *B* upon exposure to bacteria $CR = [(B_0 - B_v)/B_0] \times 100\%$.
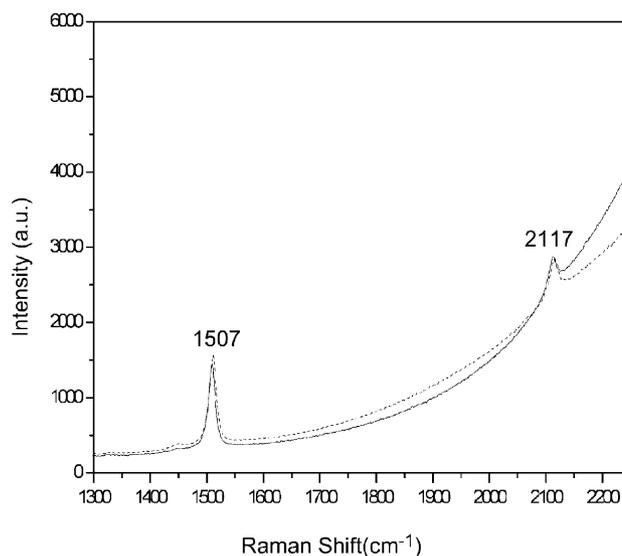

Fig. 3. Resonance Raman spectra of blue MPDA/PDA monolayer with (solid) and without (dash) the modification of CdS nano-crystallites.

The process of the affinity between *E. coli K12* and MPDA mainly includes two steps—the reversible non-specific absorption and then the irreversible specific binding. Firstly, *E. coli K12* would close to MPDA spontaneously, and the absorption was reversible. Because *E. coli K12* was negative charged, and MPDA/PDA monolayer, if was not modified with CdS nano-crystallites, was negative charged in the pH of 7.4, MPDA/PDA was inclined to repulse the approach of the bacteria. However, when MPDA/PDA monolayer was modified with CdS nano-crystallites, which were partial positive charged, the surface charge state of MPDA/PDA was changed and the electrostatic interaction between them was attraction. It would promote the affinity binding chance of *E. coli K12* to the receptor in the second irreversible specific binding process. And this promotion was in favor of enhancing the affinochromism of the MPDA/PDA in response to the bacteria. On the other hand, the color transitions in PDA are due to the changes in the effective conjugation length of the PDA backbone, and the electronic structure of PDA backbone is strongly coupled to side chain conformation [6,7,17,18]. When MPDA/PDA monolayer was modified with CdS, Cd^{2+} exchanged with H^+ of terminal carboxyl of PDA, which reduced the formation of the hydrogen bond among these terminal carbonyls of PDA [19]. Since this kind of hydrogen bond might inhibit the re-construction of MPDA/PDA bone structure, the bone structure was then less rigid and, easier to change in the second process, when this confinement was reduced.

Additionally, it was noticed that the typical energetic properties of the UV-Vis spectra did not change at all before and after the modification with CdS nano-crystallites, and similar changing trends of CR change under various incubation time were also observed (Fig. 2B). Therefore, the enhanced affinochromatic phase transition was still due to the specific binding event of *E. coli K12* to MPDA moiety but promoted by the modification with CdS.

4. Conclusion

In summary, CdS nano-crystallites played an important role not only in increasing the tropism of the *E. coli K12* to MPDA/PDA but also in decreasing the rigidity of the backbone of the PDA, which both resulted in the enhanced affinochromism. This discovery in the biomimetic membrane system offered us a new pathway to study membrane/macromolececular interaction by modifying MPDA/PDA and might be of significance in designing novel biosensor, biophysical studies, and biomedical applications. The further wok will focus on the research of photo-physical properties of MPDA/PDA-CdS monolayer in the biochemical and biophysical processes.

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