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Direct colorimetric study on the interaction of *Escherichia coli* with mannose in polydiacetylene Langmuir–Blodgett films

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Abstract

The membranes of polydiacetylene backbone decorated with mannose assembled by Langmuir–Blodgett technology can interact with *Escherichia coli*. The interactions lead to the color transition of the membranes which was readily visible to the naked eyes and could be quantified by visible absorption spectroscopy. To understand the mechanism of the chromatic transition, the affinochromism properties of polydiacetylene were examined by resonance Raman spectroscopy. The results demonstrated that the side chains of polymer backbone performed rearrangement, and the electronic structure in the polymer backbone changed from acetylene to butatriene form when the chromatic transformation from blue to red. The direct colorimetric detection by polydiacetylene membranes not only opens a new path for the use of these membranes in the area of biosensor development but also offers new possibilities for diagnostic applications and screening for binding ligand.

Keywords: Interaction; Polydiacetylene Langmuir–Blodgett film; Mannose; *Escherichia coli*

1. Introduction

Sensitive and selective molecular recognition exists in most of biological molecules, such as DNA–DNA, RNA–RNA, DNA–RNA, protein–protein, protein–sugar. It plays an important role in living activities. Furthermore certain microorganism and toxin use specific binding on the cell surface as the first step towards invasion [1].

Langmuir–Blodgett (LB) technique has been attracting attention as a tool for arranging molecules into monolayer assemblies [2]. By choosing various kinds of functional molecules, these LB films may exhibit new functions unique by the alignment of molecule in monolayers. As a promising molecule, the blue to red color transition of polydiacetylene has inspired researchers for decades [3–7]. These color changes arose from perturbations, such as temperature [8–10] and mechanical stress [11]. Especially few literatures recently, reported that a carbohydrate functiona-

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lized polydiacetylene LB film changed from blue to red upon specific binding of a biological target, it was called as affinochromism [12–14].

In order to fully study the molecular recognition role of mannose in polydiacetylene toward *Escherichia coli*, the color transition from the binding was exploited in this study. Most of the studies in the literatures so far carried out have concentrated on thermochromism [15–19]. However, the studies on the mechanism of affinochromism is very few.

In our experiment, the polydiacetylene membranes with molecular recognition function were prepared by using LB technique, subsequently visible absorption spectroscopy was applied to study the color transition of the films in response to *E. coli* and concluded that mannose can selectively recognize *E. coli*. Finally the resonance Raman spectroscopy (RRS) were used to characterize the molecular structure of different parts of the polymer and infer the affinochromism mechanism.

2. Experimental section

2.1. Materials

P-10,12-pentacosadiyne-1-*n* (3,6,9-trioxa-undecylamide) a-D-mannopyranoside (MPDA) was synthesized by Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, China, and *E. coli* K12 was obtained from School of Basic Medicine, Jilin University, China. 10,12-pentacosadiyonic acid (PDA) purchased from Farchan Laboratories (USA) was recrystallized from petroleum ether before use. All reagents used in this experiment were of analytical grade without further purification.

2.2. Preparation of LB films

The hydrophobic substrate was made by depositing a monolayer of dichlordimethylsilan (DCM) on a microslide substrate, as reported elsewhere [20]. Mixed 1.0 mM PDA/MPDA (mole ratio 20:1) dissolved in a solvent of chloroform and methanol (volume ratio 5:1) was spread on the surface of twin-compartment Langmuir trough of KSV-5000 (Made by KSV Instrument Ltd, Finland). The subphase was deionized water and its resistant was 18 MΩ cm. The film was compressed to a constant pressure of 20 mN m^{-1} at a speed of 4 mm min^{-1} at room temperature and allowed to equilibrate for 20 min (pH of subphase, 5.7). Subsequently it was irradiated with 254 nm light from ultraviolet (UV) lamp for 20 s, then the PDA/MPDA film was transferred to a hydrophobic microslide substrate covered by DCM monolayer by horizontally touching the water surface for a few seconds, respectively. The deposition ratio was 80%. The blue film was obtained and easily seen by the naked eyes as shown in Fig. 1.

2.3. Visible absorption and resonance Raman spectroscopy measurements

For the affinochromism experiments, the blue films were incubated with *E. coli* K12. Visible

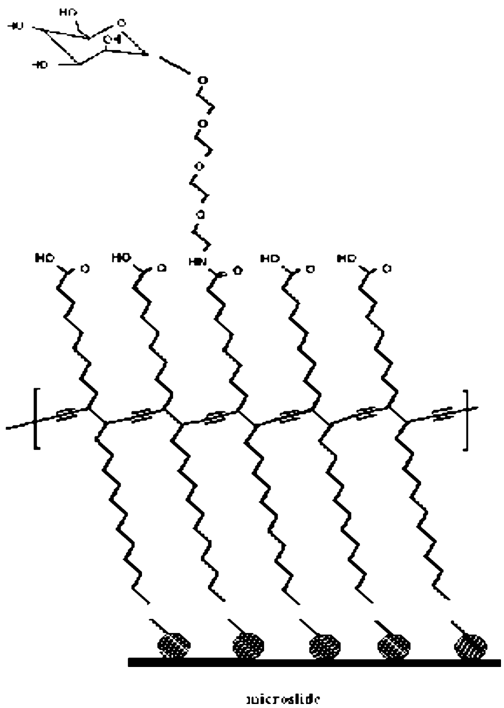

Fig. 1. Schematic diagram of the LB films used for affinochromism studies. The conjugate polymer backbones of alternating double and triple bonds constitute the chromatic detection element. The mannose is the receptor-bind ligand for *E. coli*.

absorption and RRS were carried out before and after the affinochromism.

Visible absorption spectra of the LB films were acquired at wavelengths between 400 and 800 nm on a UV-360 spectrometer. RR spectra were measured by using microlaser Raman spectrophotometer (Made in JY corporation, France) at a resolution of 1.5 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 surface of the substrate on which the PDA/MPDA was deposited was oriented at a 90° angle with respect to the laser beam. The laser beam was focused to a spot of 30 μm on the PDA films. To avoid the influence of the photoinduced thermochromism, the sample was placed in the conditions of lower temperature (5–10 °C).

3. Results and discussion

The mixed PDA/MPDA film transferred after 20 s exposure to the UV light appeared blue to the naked eyes. Its visible absorption spectroscopy characteristic of blue phase mixed PDA/MPDA is shown in Fig. 2(a). The main excitonic peak of the blue form occurred at 640 nm (blue peak) with a small absorption peak at 540 nm (red peak).

After the blue films were placed in physiological saline containing *E. coli*, the films were seen visually to go gradually from the blue to red. Correspondingly the visible absorption spectra showed a gradual decrease of the 640 nm absorption peak and a corresponding increase at 540 nm, as shown in Fig. 2(b).

In order to quantify the response of a film to a given amount of *E. coli* at different time, the visible spectrum of the film was analyzed before and after exposure to bacteria as:

$$B_x = I_{640}/(I_{540} + I_{640}) \quad (1)$$

Where B_x is defined as the intensity of the blue peak divided by the sum of the intensities of the red peak and the blue peak. The colorimetric response (CR) of a film is defined as the percent change in B_x upon exposure to bacteria, [13]

$$CR = [(B_0 - B_b)/B_0] \times 100\% \quad (2)$$

where B_0, B_b are the corresponding values of B_x before or after exposure to bacteria.

Fig. 3 displays diagrams showing the CR of PDA assemblies with or without receptor following interactions with saline solution and *E. coli*. These colorimetric data shown in Fig. 3 demonstrated the general applicability of the system for detection of mannose–*E. coli* interaction. These CR values calculated indicated that pronounced colorimetric transitions occurred only when mannose were recognized by *E. coli*. The color changes

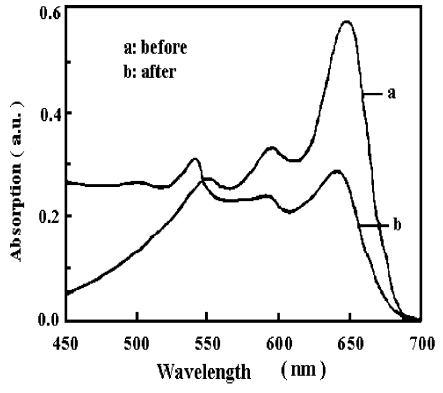

Fig. 2. Visible absorption spectra for the blue film (before) and the red film (after) obtained upon incubation with *E. coli* K12 for 15 min.

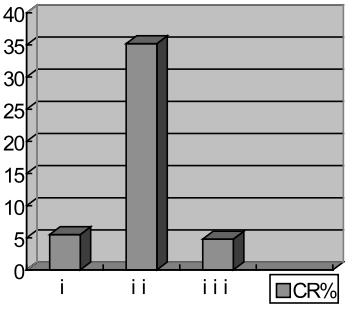

Fig. 3. Colorimetric transitions induced by different interactions (i) PDA/MPDA–saline solution; (ii) PDA/MPDA–*E. coli*; (iii) PDA–*E. coli*.

induced by nonspecific interaction between bacteria and polydiacetylene LB films were considerably smaller than the signal of the specific interaction.

Fig. 4 illustrates RR changes excited by 488 nm laser light observed during the affinochromism. The RR spectra of the blue film (presented in Fig. 4(a)) had two major bands at 1509 and 2132 cm^{-1} which may be assigned, respectively, to the stretching modes of the double and triple bonds in the polymer backbone [9]. Moreover there were two weak bands at 1575 and 2175 cm^{-1}. After the blue film was incubated with *E. coli* K12 for 5 min, the film had a slight reddish color. The RR spectrum demonstrated the decrease of the intensities at 1509 and 2132 cm^{-1}. At the same time, the intensities at 1575 and 2175 cm^{-1} increased (presented in Fig. 4(b)). After the reddish film was incubated for 10 min again, the film completely changed into red color. The RR spectrum of the resulting red film only showed the two major bands at 1575 and 2175 cm^{-1} which may be assigned, respectively, to the stretching modes of the double and triple bonds in the polymer backbone [9] (presented in Fig. 4(c)), but the bands at 1509 and 2132 cm^{-1} almost disappeared. Comparison of the three RR spectra in Fig. 4 demonstrated that (i) the bands of triple bonds and double bonds simultaneously shifted to higher wavenumbers related to their original positions during the affinochromism, (ii) the two bands (1509 and 2132 cm^{-1}) were attributable to the blue film and the two bands (1575 and 2175 cm^{-1}) were attributable to the red film.

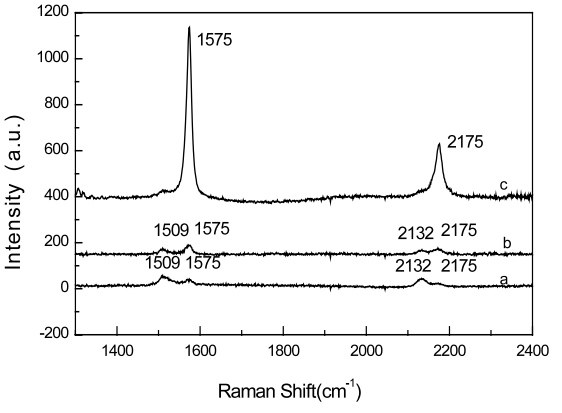

Fig. 4. RR spectra of PDA/MPDA. All spectra were recorded with 488-nm excitation. (a) Blue film prepared with 20 s UV irradiation; (b) reddish film prepared by incubating the blue film with *E. coli* K12 for 5 min; (c) red film prepared by incubating the blue film with *E. coli* K12 for 15 min.

As shown in Fig. 4, the intensity ratio of the double bonds to the triple bonds in the blue films increase from a value of 1.12 to a value of about 2.5 in the red film. It can be explained by the increase of the relative amount of the double bonds to the triple bonds during the affinochromism. Literatures [21–25] report there existed two resonance structures (presented in Fig. 5) in the polydiacetylene backbone, and the acetylene structure was energetically more favorable than the butatriene structure in the ground state of long-chain polydiacetylene molecules. From Fig. 4 we easily concluded that the increase of the relative amount of the double bonds to the triple bonds was attributable to a transformation from acetylene to butatriene.

Elucidating the physical and chemical basis of the chromatic transitions of PDA system is still an active area of research. Previous studies have suggested that color transition in polydiacetylene arose from changes in the effective conjugation length of the polydiacetylene backbone [26,27] and the electrical structure of the polymer backbone strongly coupled to side chain conformation [28,29]. So we can speculate that specific *E. coli*–mannose interaction may serve to alter the side chain conformation, causing the electrical structure of the polymer backbone change from acetylene to butatriene. The transformation of the electrical structure increases the energy level gap

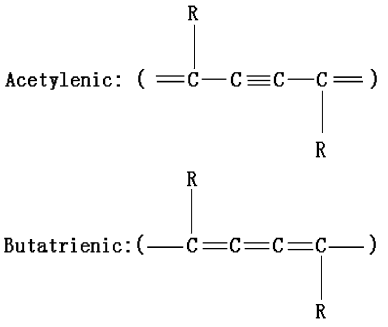

Fig. 5. Two resonance structures of the polydiacetylene.

between the π (Lumo) and π* (Homo) which causes the change of visible absorption spectra, resulting in the formation of the red film.

4. Conclusion

In conclusion, we have demonstrated that polymerized LB films provided a molecular recognition function (mannose) and a detection element (polydiacetylene backbone), and confirmed the interaction of *E. coli*–mannose and mannose was specific and could be transduced to a visible color change, readily seen with the naked eyes and quantified by visible absorption spectroscopy. In addition, on the basis of the results of RRS, we can conclude that the chromatic change results from the transformation of the electrical structure of the polymer backbone from acetylene to butatriene through the rearrangement of the side chains. The direct colorimetric detection by polydiacetylene membranes possibly not only opens a new path for the use of these membranes in the area of biosensor development but also offers new possibilities for diagnostic applications and screening for binding ligand.

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