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Colloids and Surfaces A: Physicochem. Eng. Aspects 257–258 (2005) 41–45

COLLOIDS AND SURFACES A

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Studies on poly(ethylenimine)/purple membrane multilayer films fabricated by layer-by-layer self-assembly

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Available online 19 November 2004

Abstract

In the present work, we have obtained organized PEI/PM (poly(ethylenimine)/purple membrane) multilayer films by layer-by-layer alternate adsorptions with positively charged polycation PEI and negatively charged purple membrane fragments. The results observed by UV–vis spectroscopy show that each transfer amount of PM fragments onto the substrate is equal and the characteristic absorption bands of bacteriorhodopsin (bR) are still preserved. AFM and SEM analysis investigated the morphology and cross-section of PM films, respectively. The photocurrent peaks of $(PEI/PM)_6$ multilayer films corresponding to light-on and light-off are ca. 440 and 250 nA/cm^2.

Keywords: Bacteriorhodopsin; Purple membrane; Poly(ethylenimine); Layer-by-layer assembly; Films

1. Introduction

Bacteriorhodopsin (bR) is the key protein imbedded in purple membrane (PM) isolated from *Halobacterium salinarum* [1] and functions as a light-driven proton pump [2]. Upon absorption of light, the retinal isomerizes from the all-*trans* to the 13-*cis* conformation [3]. This triggers bR molecule to undergo structural change [4], and protons are pumped from the cytoplasm through the halobacterial cell membrane to the outer medium, and a transmembrane proton gradient is generated [5]. The phenomenon of an electrical potential across a membrane or an electrical current through a membrane responding to generating vectorial charge is called photoelectric effect. Recently, more and more attention has been drawn on fabricating biology devices with bR with unique character of differential photoelectric response under excitation. The photoelectric property can be utilized for photoelectric picture converters, such as motion sensors [6], and artificial retinas [7].

Performance of electric devices of bR is greatly dependent on fabrication of bR films. Over the past two decades, a number of methods have been developed to fabricate the PM films, such as free deposition, Langmuir–Blodgett (LB) deposition [8], electric field sedimentation (EFS) [9], electrostatic layer-by-layer (LBL) adsorption [10], antigen–antibody molecular recognition [11], polymer used as immobilizing matrices [12], and so on. Of all these methods, LB deposition is a convenient method and has an attractive potential because of a variety of polycations used for selection. LB assembly process based on electrostatic interaction is a unique technique that presents a new approach to the formation of ultrathin films by adsorption of consecutively alternating polyelectrolytes [13,14]. The most suitable advantage of the LB self-assembly to our work is it can be easily applied to water-soluble bR protein without mixing it into soya lecithin used in LB films.

Herein, PEI was used as a polycation to fabricate the PEI/PM multilayer films. The characteristics of PEI/PM multilayer films were investigated by UV–vis spectrophotometer (Hitachi U-2800), atomic force microscopy (AFM, NanoScope IIIa) and scanning electron microscope (SEM, Hitachi S-4200). The photocurrent responses were studied with the photoelectric measurement equipment (home-made).

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doi:10.1016/j.colsurfa.2004.10.075

2. Materials and method

The strain of *H. salinarum* was the ET1001 (also known as JW-1) strain. Growth, purification and sample preparation of bR embedded in the PM were implemented in our lab according to the standard method [15] with certain improved procedures [16]. The absorption ratio of $A_{278\,nm}/A_{566\,nm}$ was 2.1, which suggested a good quality of our sample [17]. In this study, the PM fragments with the concentration about 0.5 mg/mL of bR were ultrasonic comminuted about 200 s (power is 300 W), and the pH of PM suspension was adjusted to 9.0 using Tris–HCl buffer. Fig. 1 is an absorption spectrum of PM fragment dissolved into tri-distilled water (pH 9.0). The absorption maximum is still observed at 566 nm, which is similar to that found in neutral conditions. This indicates that the spectral properties of PM fragments are not changed in alkaline medium. Poly(ethylenimine) (PEI) was purchased from Aldrich Chemical Co. and was used without further purification. Before experiment, PEI was dissolved into tri-distilled water containing 0.5 mol/L NaCl, with the concentration was 2 mg/mL.

Solid supports used for depositing the PEI/PM multilayer films: quartz slides (10 mm × 40 mm) were used for UV–vis absorption measurements; ITO glass slides (10 mm × 40 mm) were used for SEM analysis and photoelectric property measurements, and mica slices were applied for AFM analysis. In order to get a net negative charge surface, all the solid supports were treated according to the description of Ref. [11]. The films were irradiated with 30 mW/cm^2 of green light from a 300 W xenon lamp through a 540 nm long-pass filter and an adiabatic glass. Other equipments were picoammeter (0.1 nA range) and a digital storage oscilloscope (20 MHz, Gold Star). The PM-immobilized ITO conductive glass was used as working electrode of a photo electrochemical cell and Pt was the counterelectrode. The distance between two electrodes was ca. 8 mm. KCl (0.5 mol/L) aqueous solution was applied as supporting electrolyte. All the measurements were carried out at room temperature in air. The effective illumination area of PEI/PM films used in measurement was about 0.3 cm^2 and the photoelectric response value was averaged 10 times to minimize the error.

2.1. Fabrication of PEI/PM multilayer films

A solid support after negatively charged treatment was immersed into PEI aqueous solution (2 mg/mL, pH 6.0) for 5 min and was rinsed with tri-distilled water, and then it was dried with nitrogen flow. The modified slide was immersed into PM suspension (0.5 mg/mL bR, pH 9.0) for 5 min and was rinsed with tri-distilled water, then dried it with nitrogen again. This process was repeated until the required films were obtained. All adsorption steps were carried out at room temperature.

3. Results and discussion

3.1. Spectra analysis

In neutral and alkaline environments, cytoplasmic side of PM fragments is known to have more negative charges than extracellular side of it. Due to this structure, PM fragments can be easily adsorbed onto a positive surface. Fig. 2 shows the UV–vis absorption spectra of the alternative PEI/PM multilayer when every bilayer was assembled onto the substrate. From this figure, it can be seen that the characteristic absorption peaks at 278 and 560 nm are still observed distinctly. The absorptions of the bands from 555 to 560 nm are almost equal, which is about 10 nm blue shift comparing with the peak absorption of PM fragment suspension at 566 nm because of a dehydration effect on the Schiff base of the retinal chromophore in bR in the dried PEI/PM films. The result of blue shift is consistent with bR LB films [18], while in the situation with sufficient water, a red shift will be observed and the peak absorption can be recovered to that of PM solution [19]. The inset makes it clear that absorbance at 560 nm increases proportionately with increase of the number of PEI/PM bilayer.

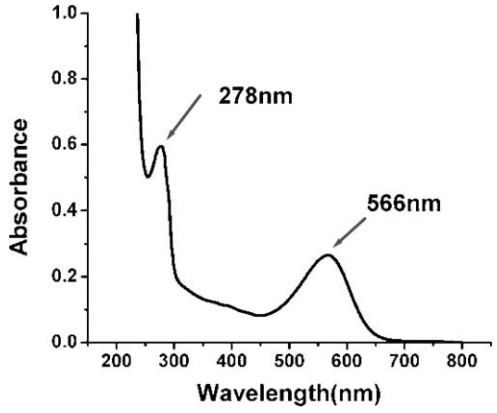

Fig. 1. UV–vis absorption spectrum of PM fragments dissolved in tri-distilled water (pH 9.0, adjusted with Tris–HCl buffer).

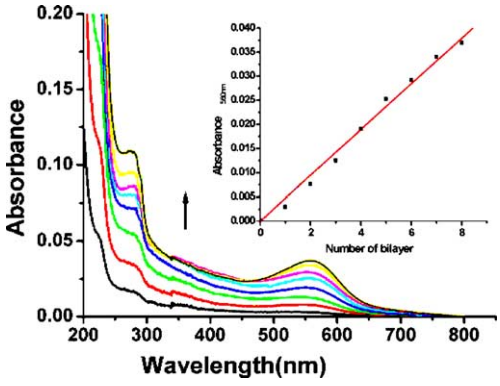

Fig. 2. UV–vis absorption spectrum of PEI/PM multilayer films. The curves, from bottom to top, represent the adsorption of 1–8 bilayer of PEI/PM multilayer films, respectively. The inset indicates the increase of absorbance of 560 nm with the number of bilayer.

It suggests that each bilayer of PEI/PM multilayer films has the equal thickness at each deposition step, and the transfer amount of bR in PM fragments is equal.

3.2. AFM and SEM studies

Because atomic force microscopy (AFM) studies can provide further information about the surface morphology of PEI/PM multilayer films down to the nanometer scale, so we investigated the surface morphology of each bilayer of PEI/PM multilayer films by AFM in tapping mode. It can be seen from Fig. 3a that polycation PEI is uniformly absorbed onto mica wafer and the PEI layer surface is smooth on the whole but with little bulge, which is consistent with the description in literature [20]. Fig. 3b–d shows the morphology images of one-, two- and four-bilayer of PEI/PM multilayer films (PM is the outer layer), respectively. In brief, PM absorbed to the PEI surface is distributed uniformly and packed compact, only a multitude of small domains can be observed, which are probably due to the different size of PM fragments and measly overlap of PM. Comparing with the surface topography of bR film fabricated by LB and EPS technique, less aggregates of PM fragments are found by LBL assembly and the uniformity and the degree of bR orientation are much better [21,22]. We evaluated the surface roughness of four images above moreover. We report the roughness in terms of their mean roughness (R_a) values by image analysis. For each film surface, R_a values describe the roughness of that structure. We can see the roughness is slightly increased with a greater number of bilayer.

Fig. 4 shows the image of cross-section of four- and eight-bilayer PEI/PM films, respectively, studied with scanning electron microscope (SEM). It can be seen from Fig. 4 that the cross-section was divided into three parts, which are glass, ITO conductive layer and PEI/PM films layer. The thickness of PEI/PM films is an important factor to study the devices of bR, and here we obtain the thickness of each PEI/PM bilayer by analysis of the SEM cross-section image. The thickness

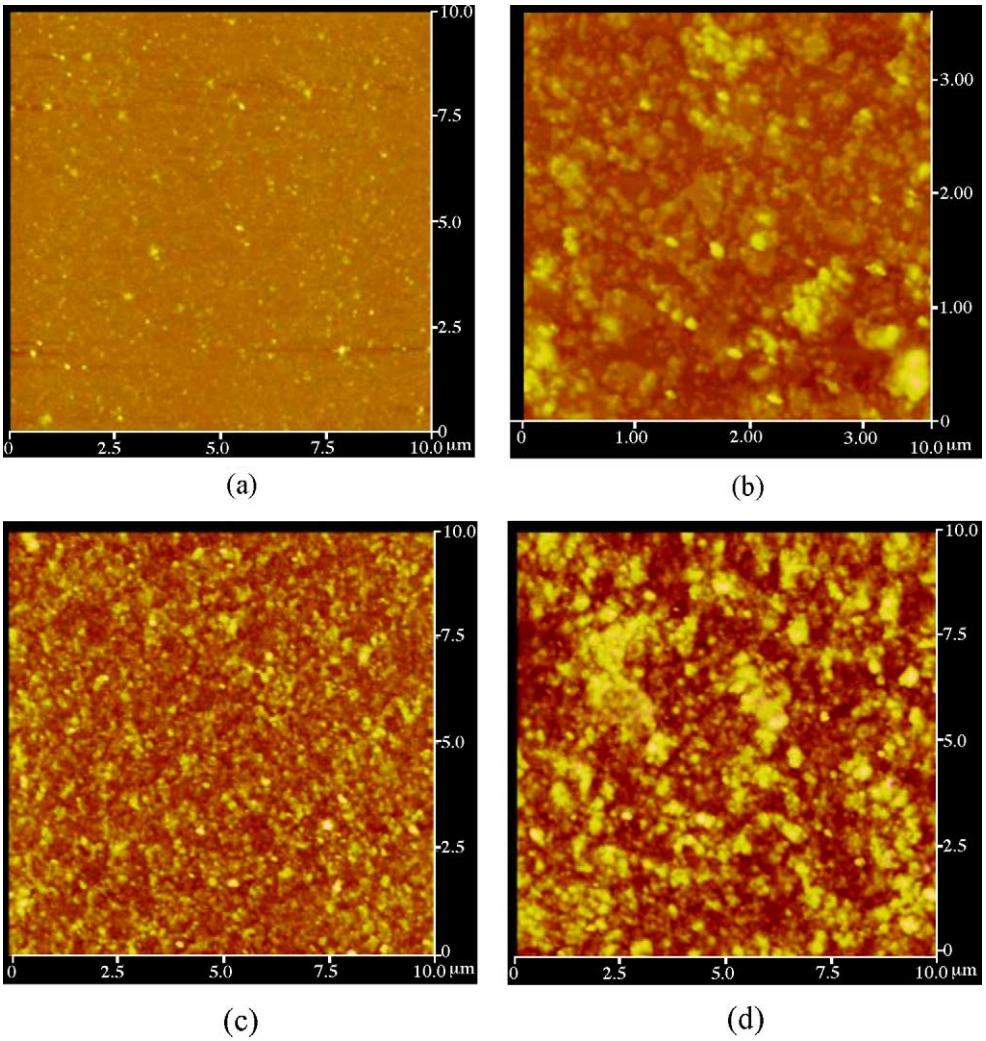

Fig. 3. AFM images of the PEI/PM multilayer films: (a) PEI layer adsorbed onto mica wafer (R_a = 0.221 nm); (b) one layer of PM adsorbed onto PEI layer (R_a = 3.509 nm); (c) two bilayer of PEI/PM multilayer films (R_a = 5.400 nm); (d) four bilayer of PEI/PM multilayer films (R_a = 12.327 nm).

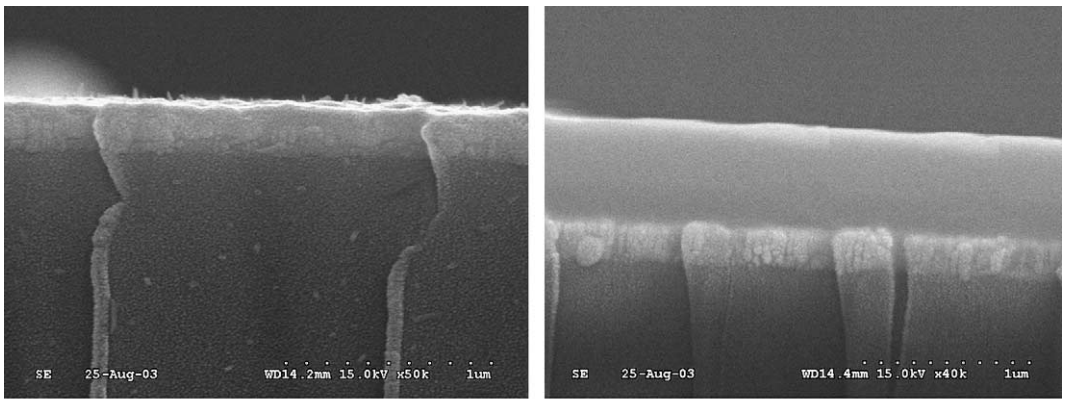

Fig. 4. SEM images of cross-section of PEI/PM multilayer films: (a) four bilayer of PEI/PM multilayer films; (b) eight bilayer of PEI/PM multilayer films.

of each PEI/PM bilayer is calculated to be 60 ± 5 nm that is accordant with the value of the films fabricated by LB method [23].

3.3. Photoelectric properties

The most significant property of bR is its differential photoelectric response to change of light intensity, which is desirable for bioelectronic device applications. Under a constant light intensity, the transient photocurrent undergoes rapidly and offset down to the base level. Moreover, the magnitude and quantum efficiency of the differential photocurrent generated from bR films are quite dependent on the orientation degree of bR in the films and the environment of the system of a circuit. Fig. 5 shows a typical photoelectric response profile of the PEI/PM multiplayer films. Positive and negative photocurrents are generated when light is switched on and off, respectively, following with rapid relaxation to the base level. The characteristic differential photocurrent is attributed to the proton release and uptake occurring in the photoexcited bR molecules [24]. It is observed that the signal shape is very similar to the typical photocurrent of the PM ultrathin films fabricated by LB and EPS technique [22,25,26]. The photocurrent peak of light-on and light-off is about 440

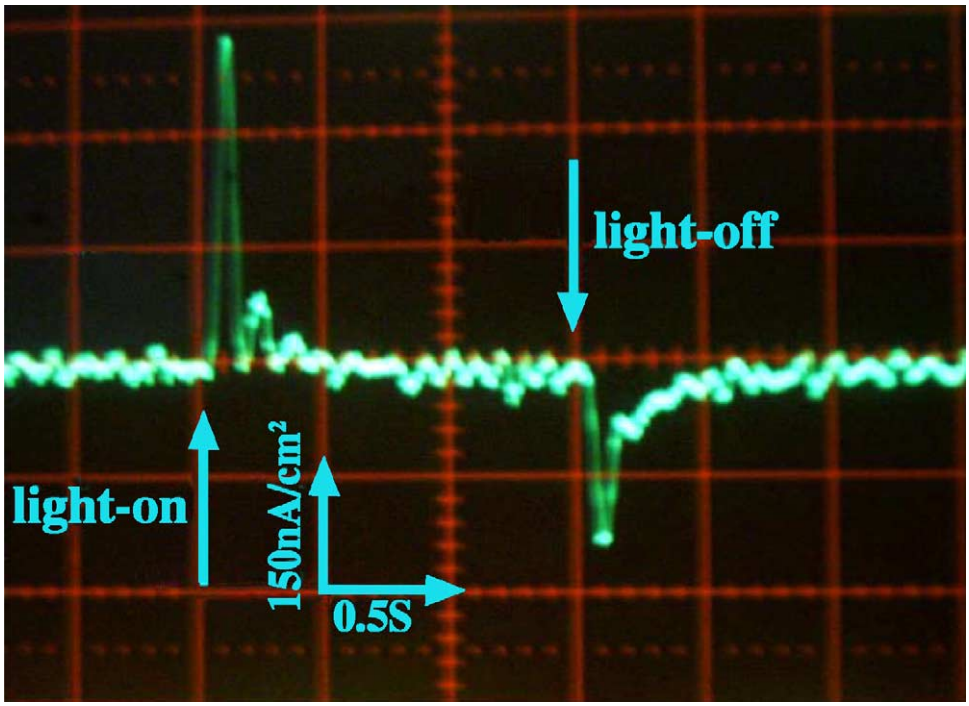

Fig. 5. A typical photoelectric response of the $(PEI/PM)_6$ multilayer films upon the light illumination.

and 250 nA/cm^2, respectively, and the decay time is about 60 ms that is much faster than that of LB and EPS fabricated films, which can be explained for the better uniformity and orientation degree.

4. Conclusion

Well-oriented PEI/PM multilayer films can be successfully fabricated by layer-by-layer electrostatic adsorption. The result studied by UV–vis absorption spectra shows that the characteristic absorption band of bR is still preserved and the process of deposited PEI/PM bilayer can be well reproducible. AFM and SEM investigations show that PM fragments are more densely packed in PEI/PM multilayer films than that in PM films fabricated by LB or electric field sedimentation, and the thickness of each bilayer of PEI/PM multilayer films is ca. 60 ± 5 nm. Photoelectric response of PEI/PM multiplayer films confirms the biological integrity and activity of bR are maintained. So PEI/PM multilayer films oriented by layer-by-layer deposition is a promising usable material for the fabrication of bR nanobiomolecule devices.

Acknowledgements

The authors appreciate the financial support of this work by Nanometer Devices and their Fundaments, Important Program of Knowledge Innovation Project of Chinese Academy of Sciences (KJCX1-SW-07).

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