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Short Communication

Langmuir-Blodgett Film of Phycobilisomes from Blue-Green Alga Spirulina platensis

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Abstract The phycobilisomes were isolated from blue-green alga *Spirulina platensis*, and could form monolayer film at air/water interface. The monolayer film of phycobilisomes was transferred to newly cleaved mica, and coated with gold. Scanning tunneling microscope was used to investigate the structure of the Langmuir-Blodgett film of phycobilisomes. It was shown that phycobilisomes in the monolayer arrayed in rows with core attaching on the substrate surface and rods radiating towards the air phase, this phenomenon was similar to the arrangement of phycobilisomes on cytoplasmic surface of thylakoid membrane *in vivo*. The possible applications of the Langmuir-Blodgett film of phycobilisomes were also discussed.

Key words *Spirulina platensis*; phycobilisome; Langmuir-Blodgett film; scanning tunneling microscope (STM)

The phycobilisomes (PBS) are the photosynthetic light-harvesting complexes in blue-green algae and red algae[1-5]. They have been exhaustively studied with biochemical, biophysical, and electron microscope methods, especially those from blue-green algae[6]. Phycobilisomes are composed of several kinds of phycobiliproteins and colorless polypeptides, which assembled in specific configuration for optimized energy transfer to downstream photosynthetic complexes[7,8]. According to their spectroscopic properties, phycobiliproteins are divided into phycoerythrin (PE), phycoerythrocyanin (PEC), phycocyanin (PC) and allophycocyanin (APC). Because of their physical and spectroscopic properties, phycobiliproteins are widely used as labeling reagents for a variety of fluorescence detection applications, including flow cytometry, and are attractive as one of the most promising molecules for bioelectronics[9,10]. The photosynthetic light harvesting pigment complexes in green algae and higher plants were located in thylakoid membrane, otherwise, the PBS is arranged in rows on thylakoid surface with the core attached to the membrane surface[4].

In our previous work, it was shown that the phycobilisome in *Spirulina platensis* is composed of APC and C-phycocyanin (C-PC). C-PC is assembled into the rod of the phycobilisome, and APC is stacked in the core of the phycobilisome. The rods radiated from the core to different directions, and the APC-rich phycobilisome core is then attached to the photosynthetic membrane, allowing the efficient light energy transfer to photosynthetic system II (PSII)[11]. The three dimensional structure of the C-PC was observed with STM[12]. We has also found that the water-soluble R-PE could self-assembly into rod-like structures when absorbed on the surface of HOPG, and could form two-dimensional Langmuir-Blodgett film at the air/water interface, its structure was observed by STM[13]. Facci *et al*.[14] studied the structure of monolayer of reaction center of photosynthetic bacterium and its property of light-electricity conversion with STM. But to date, there was no report on the ability of phycobilisomes to form two-dimensional film by LB technique.

In this paper, it was found that phycobilisomes could form two-dimension Langmuir-Blodgett film at the air-water interface.

1 Materials and Methods

1.1 Isolation of phycobilisomes

The phycobilisomes were isolated from blue-green

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alga Spirulina platensis according to the procedure of Gantt and Lipschultz[1] with some modifications. The fluorescence property at 77 K showed that the isolated PBS was intact.

1.2 Preparation of LB film

The monolayer of PBS was prepared on Sixing film deposition system (Jilin University, China) with a surface area of approximate 648 cm^2. Deionized ddH_2O (pH 5.6) was used as the subphase. π-A curve measurement was carried out by spreading a 2% ethanol/water solution containing about 0.4 g/L PBS onto the subphase surface, and ethanol solvent was allowed to evaporate for 15 min before compressing the monolayer at a rate of 0.5 cm^2/s. Surface pressure was measured with Wilhelmy plate. Monolayer was compressed to a surface pressure of 15 mN/m, and was allowed to stabilize for at least 40 min before dipping down the mica matrix. The mica was a suitable substrate for transferring PBS monolayer because it has a negatively charged surface, which was similar to the thylakoid membrane surface. The lifting speeds were 5 mm/min upward and 15 mm/min downward. The transfer ratio for PBS in the upward collection was approximately 0.85, and no deposition took place during downward motion. In order to enhance the electroconductivity of the film, the mica matrix onto which PBS monolayer was deposited was coated with gold and the thickness of gold film should be controlled as thinner as possible so as to minimize its interference. However, a continuous gold layer should meanwhile be formed to have good electroconductivity. In order to obtain good STM images, only one layer of PBS monolayer was transferred onto the mica matrix.

1.3 STM experiments

STM experiments were carried out in ambient environment with a CSTM-9100 STM machine (manufactured by Institute of Chemistry, the Chinese Academy of Sciences). STM measurement was performed with normal constant current mode, using tungsten tips made by electrochemical etching. All STM images were presented from raw data without any smoothing and filtering.

2 Results

Fig. 1 is the 77 K fluorescence spectrum of PBS from *Spirulina platensis* excited at 580 nm. The major emission peak was at 685 nm, typical of intact PBS, and another minor maximum at 623 nm. PBS showed F685 excitation maxima at 617 nm and 650 nm, which were the absorption maxima of C-PC and APC, respectively. From Fig. 1, it could also be deduced that C-PC was the major source of F685. These results indicated that the isolated PBS from *Spirulina platensis* was intact.

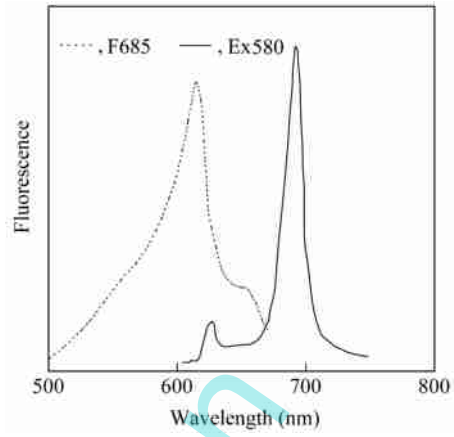

Fig. 1 Fluorescence spectra of phycobilisomes isolated from Spirulina platensis **at 77 K**

PBS is a water-soluble phycobiliprotein complex. But usually its pure aqueous solution was not satisfactory for spreading in the preparation of LB film. Thus, it was crucial to find a suitable spreading solvent, in which this water-soluble compound could spread to form monolayer, but not denature in the meantime. Results showed when PBS was dissolved in 2% (*V*/*V*) ethanol/water solution, it can keep intact, and the proteins will not denature (data not shown).

Fig. 2 was the surface pressure-area isotherm at room temperature of PBS monolayer at the air/water interface. From Fig. 2, it could be resulted that PBS could form monolayer when they were dissolved in 2% ethanol/water spreading solution. Usually, water-soluble molecule are apt to be desorbed from air/water interface to subphase (water), so the monolayer of these compounds was not stabilized. Since the interfacial concentration of PBS used in our experiment was far less than the limiting interfacial concentration of proteins, the desorption can be

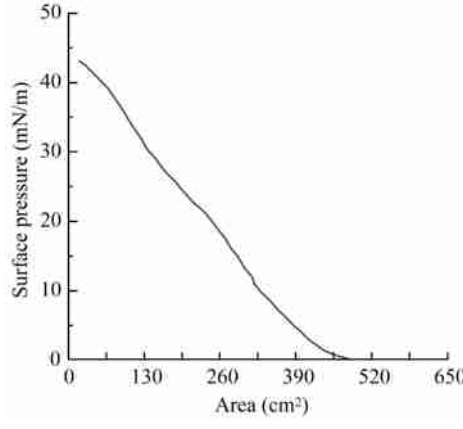

Fig. 2 Surface pressure-area isotherm of phycobilisome monolayer at the air/water interface

The quantities of phycobilisomes deposited were 32 μg. The subphase was composed of deionized ddH_2O (pH 5.6). All measurements were carried out at room temperature.

neglected. The change in the area of PBS monolayer at the air/water interface with time at constant surface pressure of 15 mN/m was presented in Fig. 3. From the illustration, it can be found that the area of PBS monolayer at the air/water interface hardly changed within 3 h after 15 min post-preparation, indicating that the monolayer of PBSwas very stable once the monolayer was formed at the air/water interface.

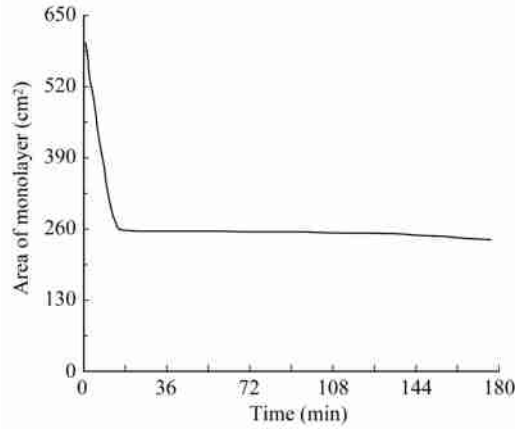

Fig. 3 Changes in areas of phycobilisome monolayer with time

One PBS monolayer was transferred onto the newly cleaved mica, coated with gold and its structure was observed by STM. The STM images of PBS LB film were shown in Fig. 4. It was shown that the phycobilisomes arranged orderly in the LB film, most PBS arrayed in rows [Fig. 4(A), shown along the line]. Single PBS could be distinguished, with the core of PBS attached to mica surface, and rods of PBS radiated towards the air phase [Fig. 4(B), shown by the arrows], which was in agreement with its arrangement on thylakoid membrane surface *in vivo*. No obvious defect was observed in the scanning area of 830 nm ×830 nm. These results showed that intact PBS had good ability to form two-dimensional film, and the film could easily be transferred onto mica matrix.

3 Discussion

Due to their stability, high fluorescence yield, large stokes' shifts between absorption and emission[9], the phycobiliproteins are widely used as labeling reagents for a variety of fluorescence detection applications, including flow cytometry[10]. Recently, the stabilized phycobilisomes were used as fluorochromes for the detection, which had far greater fluorescence intensity per binding event than a single phycobiliprotein molecule[15,16]. Chronakis *et al*.[17] reported that the protein-pigment complexes isolated from the phycobilisome of *Spirulina platensis* are quite capable of forming Langmuir-Blodgett films. The protein layer spreading at the air/aqueous interface also

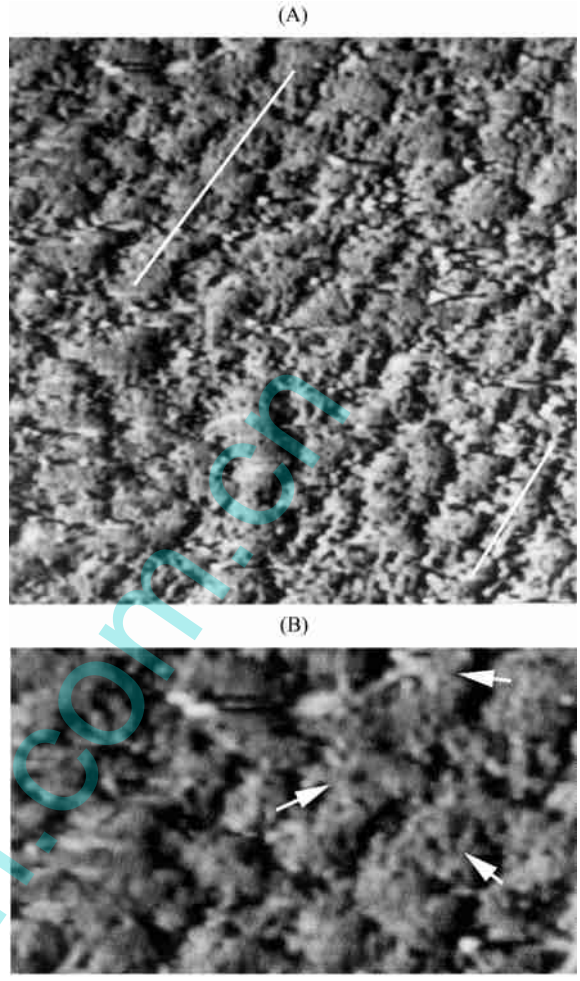

Fig. 4 STM image of phycobilisome monolayer
(A) Scan area: 830 nm ×830 nm. (B) Scan area: 460 nm ×260 nm. I_t = 0.60 nA; V_{bias} = 510 mV.

has a higher collapse pressure than the common food proteins. We also found that the R-phycoerythrin from marine red alga *Polysiphonia urceolata* can be easily prepared for two-dimensional film with Langmuir-Blodgett technique[13]. In this paper, it was found that PBS could also form two-dimensional Langmuir-Blodgett film at the air/water interface. With special property of photophysics and photochemistry, phycobiliproteins and phycobilisomes might be acted as a kind of useful materials for crystallization electronics and bioelectronics research. Moreover, STM could be used to study the property of light-electricity conversion and to manipulate the molecules or complexes in the Langmuir-Blodgett film with STM tip[14,18]. On the base of this research, the Langmuir-Blodgett films of phycobilisomes might finally be used as functional organic biological materials for data storage and molecular switch.

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钝顶螺旋藻(Spirulina platensis)藻胆体 Langmuir-Blodgett 膜

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摘要 从钝顶螺旋藻中分离制备完整藻胆体, 然后滴加于空气/水界面上, 应用 LB 膜技术制备藻胆体 LB 膜。结果表明, 藻胆体在空气/水界面上具有很好的成膜性能。将藻胆体 LB 单层膜转移到刚揭开的云母表面, 喷一层金, 然后用扫描隧道显微镜观察。结果表明, 藻胆体在 Langmuir-Blodgett 膜中的排列方式与其在体内类囊体膜表面的排列方式类似, 一排排聚集在一起, 然后排列成膜。藻胆体的"核"吸附在云母表面, 而藻胆体的"杆"伸向外面。由于钝顶螺旋藻易于规模化培养, 藻胆体容易批量制备, 加之藻胆体具有的独特的光物理、光化学特性和良好的成膜性能, 以及本身就是纳米量级的颗粒(5070 nm), 预示着藻胆体在纳米光电子器件中具有很好的应用前景。

关键词 钝顶螺旋藻; 藻胆体; Langmuir-Blodgett 膜; 扫描隧道显微镜(STM)

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