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Short Communication

In vitro Assembly of R-phycoerythrin from Marine Red Alga Polysiphonia urceolata

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Abstract Scanning tunneling microscope was used to investigate the *in vitro* assembly of R-phycoerythrin (R-PE) from the marine red alga *Polysiphonia urceolata*. The results showed that R-PE molecules assembled together by disc-to-disc while absorbing on HOPG surface, which just looked like the rods in the phycobilisomes. When the water-soluble R-PE was dissolved in 2 % ethanol/water spreading solution, they could form monolayer film at the air/water interface. Similar disc-to-disc array of R-PE was constituted in the two-dimensional Langmuir-Blodgett film by the external force. It could be concluded that, apart from the key role of the linker polypeptides, the *in vivo* assembly of phycobiliproteins into phycobilisomes is also dependent on the endogenous properties of phycobiliprotein themselves.

Key words R-phycoerythrin; *in vitro* assembly; scanning tunneling microscopy (STM)

Phycobilisomes (PBS), the light-harvesting antennae of blue-green algae and red algae, are supramolecular, highly structured protein complexes located on the thylakoid surface[1]. They are primarily composed of chromophore-bearing phycobiliproteins absorbing light over a wide spectral range[1]. Phycobiliproteins in blue-green algae and red algae are composed of $(\alpha\beta)$-monomer. During the last decade, the crystal structures of several phycocyanin (PC), phycoerythrin (PE), allophycocyanin (APC) have been determined by X-ray diffraction[2—7]. These results revealed that all the phycobiliproteins have very similar three-dimensional structures. Three $(\alpha\beta)$-monomers are arranged around a 3-fold symmetry axis in the trimer $(\alpha\beta)_3$, while two trimers are assembled by disc-to-disc into the hexamer $(\alpha\beta)_6$. In addition, PBS also contain a minor portion of proteins, most of which do not bear chromophores, and are referred to as "linker polypeptides" whose function is to make phycobiliproteins assemble as PBS and stabilize the supramolecular complex *in vivo*[1].

The assembly of phycobiliprotein discs into phycobilisomes is dependent upon the presence of different functional linker polypeptides. The roles of the linker polypeptides in the aggregate formation and the fine-tuning of the absorption characteristics of phycobiliproteins have been extensively studied[8—11], and the structure model of the phycobilisomes in *Mastigocladus lainosus* was proposed[12]. However, apart from the function of the linker polypeptides, the phycobiliprotein itself might play an important role in the assembly of the phycobilisomes. Because, although there were phycobiliproteins in the Cryptophyceae and some of the Dinophyceae, phycobolisomes did not exit, and the subunit composition of the phycobiliproteins was different from that of the blue-green algae and red algae[1]. The observation of interaction between the phycobiliprotein molecules could give the evidence for the above suggestion. The three dimensional structure of the C-phycocyanin (C-PC) and phycobilisomes in *Spirulina platensis* was observed with STM[13,14]. In this paper, STM was used to investigate the spontaneous behavior of pure R-phycoerythrin (R-PE) without linker polypeptides while adsorbing on the newly cleaved highly oriented pyrolytic graphite (HOPG) surface, and then, we

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report the abilities of R-PE to form two-dimensional orderly films by Langmuir-Blodgett (LB) technique and their structural observations.

1 Materials and Methods

1.1 Isolation of R-PE and absorption on the HOPG surface R-PE was purified from marine red alga *Polysiphonia urceolata*. The phycobiliproteins were extracted by autolysis in distilled water, and then by fractional precipitation with ammonium sulphate from high to low concentration (55 %, then 50 %—45 %). The crude sample was separated through a hydroxylapatite column, washed with 30 mmol/L phosphate buffer (pH 6.8, 0.2 mol/L NaCl). The collected R-PE was finally purified through a Bio Gel P-300 column (Bio-Rad, Richmound, UA), washed with 50 mmol/L sodium-phosphate buffer (pH 6.8, 0.1 mol/L NaCl). The absorption spectrum was determined with Shimadzu UV-240 spectro photometer at room temperature.

The R-PE sample solution was dialyzed against 5 mmol/L phosphate buffer (pH 6.8) for 24 h and diluted with distilled water. Then, 5 μl diluted sample solution was dropped on freshly cleaved HOPG surface, and then stayed on it for 20 s for Fig. 2(A) and 1—2 min for Fig. 2(B) in air at room temperature. The excess solution was removed with filter paper. The concentration of R-PE was approximately 5 mg/L in Fig. 2(A) and 20 mg/L in Fig. 2(B), respectively.

1.2 Preparation of LB films All monolayers were prepared on Sixing Film Deposition System (Jilin University, China) with a surface area of approximate 648 cm^2. Triple distilled and adjusted water (pH 5.6) was used as subphase. π-A curve measurements were carried out by spreading a 2 % ethanol/water mixture containing about 0.3 g/L R-PE onto the subphase surface, and ethanol solvent was allowed to evaporate for a period of 15 min before the compression of the monolayer at a rate of 0.5 cm^2/s. Surface pressure was measured with Wilhelmy plate. Monolayer was compressed to a pressure of 15 mN/m, and was allowed to stabilise for a period of at least 40 min before dipping down the mica matrix. The lifting speeds were 5 mm/min upward and 15 mm/min downward. The first layer was allowed to dry for 50 min to ensure good contact between the mica matrix and the monolayer. For the subsequent layers, the time for standing at the down and up positions were 1 and 12 min, respectively. The transfer ratio in the upward collection was approximately 0.95, and no deposition took place during downward motion. Newly cleaved mica were used as the matrix for the preparation of LB films of R-PE. In order to enhance the electroconductivity of the films, the matrix onto which R-PE monolayer was deposited was coated with gold. The thickness of gold film was controlled as thinner as possible so as to minimize the interference of gold film, however, a continuous gold layer should meanwhile be formed so as to have good electroconductivity. In order to obtain good STM images, three layers of R-PE were transferred onto the mica.

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

2 Results and Discussion

Fig. 1 was the absorption spectrum of R-PE isolated from marine red alga *Polysiphonia urceolata*. The major absorption peaks were located at 498 nm, 545 nm and 565 nm, which was associated with the reported results of R-PE[1]. The absorbent ratio of $A_{565}/A_{280} > 4$ suggested that the purity of isolated R-PE was good. It was confirmed by the SDS-PAGE electrophoresis that there was no linker polypetide in the isolated R-PE solution.

The actual distribution of the sample on substrates greatly depends on the concentration and size of biological objects, as well as the time allowed for adsorption. We have succeeded to observe the structure of individual C-PC molecules with STM. The concentration of C-PC solution was 5 mg/L, and the time for adsorption was 20 s. Here the concentration of R-PE in the solution was raised to 20 mg/L, R-PE solution was dropped on HOPG surface and stayed on it for 1—2 min. In this case, all most of R-PE molecules aggregated together, and few individual molecules were found on the HOPG surface

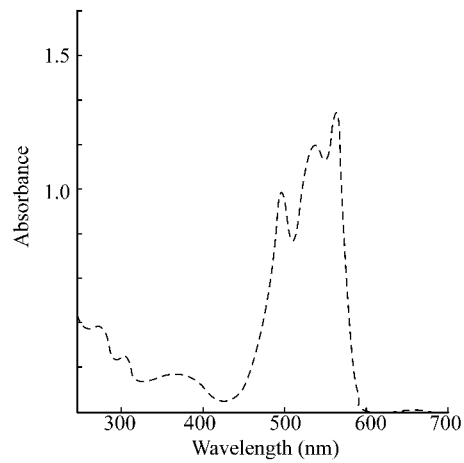

Fig. 1 The absorption spectrum of R-PE from marine red alga Polysiphonia urceolata

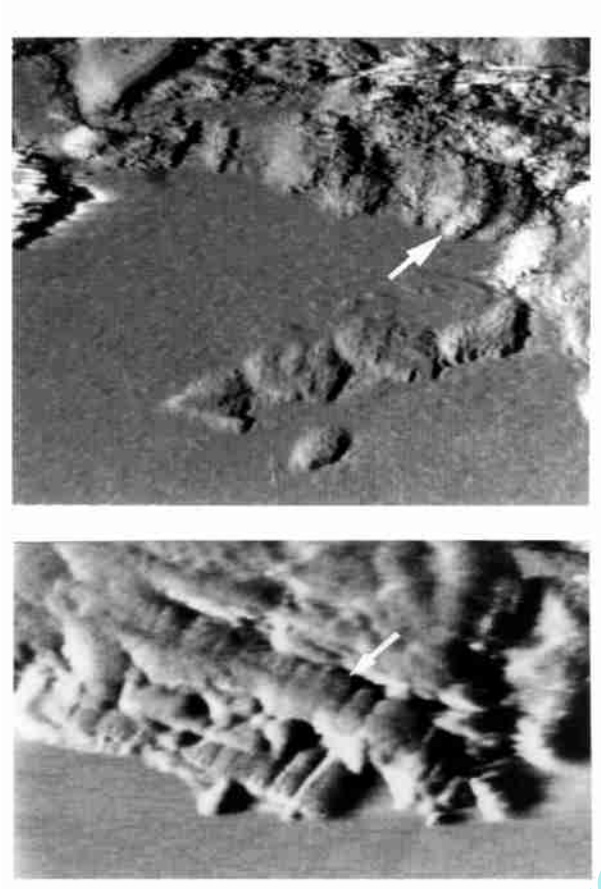

Fig. 2 STM images of R-PE
It = 0.48 nA, Vbias = - 235 mV. Scan area: (A) 128 nm × 128 nm, (B) 55 nm × 75 nm.

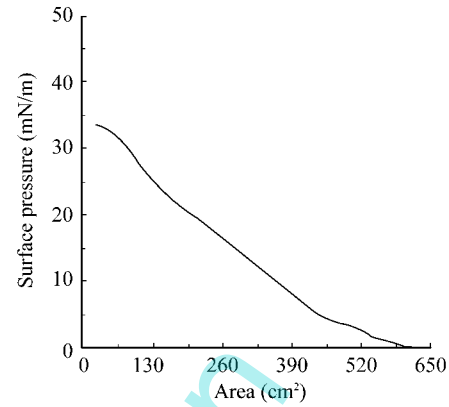

Fig. 3 The π-A curve of R-PE monolayer

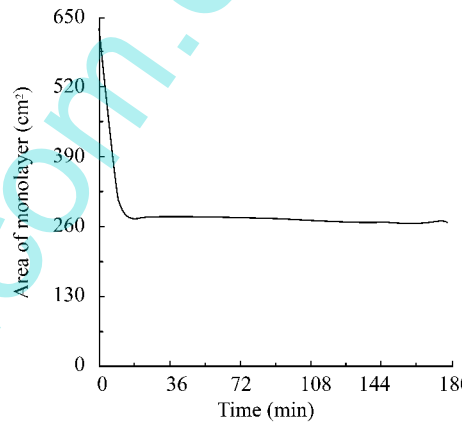

Fig. 4 Changes in area of R-PE monolayer with time

from the STM image shown in Fig. 2. In some area, R-PE molecules assembled by disc-to-disc to form more regular aggregate which was just like the native rod in phycobilisomes. Therefore, the aggregation state *in vitro* verified the rod model structure of phycobilisomes. The thickness of the disc in the rod was about 10 nm. The diameter of the rod was approximately 40 nm. The aggregation of R-PE was $(\alpha\beta)_6\gamma$ *in vivo*, every one disc in the rod might be referred as one $(\alpha\beta)_6\gamma$.

In order to further study the self-organizing ability of R-PE *in vitro*, LB technique was used to prepare LB film of the protein. As R-PE is a water-soluble protein, small amount of ethanol was added to improve the performance of the film deposited [R-PE was not denatured while dissolved in 2% ethanol/water solution (data not shown)].

Fig. 3 was surface pressure-area isotherms of R-PE monolayers at the air/water interface. R-PE formed monolayers when they were dissolved in 2% ethanol/water spreading solution. Due to the fact that the interfacial concentrations of R-PE used in our experiments were far less than the limiting interfacial concentration of proteins (0.78 m^2/mg), the desorption may be negligible. The monolayer formed under our experiment conditions was stable. It could seen from Fig. 4 that, at constant surface pressure of 15 mN/m, the areas of the monolayers hardly changed within 3 h. In addition, it was proved that the pretreated mica was a suitable matrix for transferring R-PE monolayer, which might be related to the fact that the mica has a negatively charged surface, which was similar to the thylakoid membrane surface.

Fig. 5 was the STM image of R-PE LB film. From the image, it could be observed that R-PE molecules could form a regular film, no obvious defects were found in the scan area of 480 nm × 480 nm. Therefore, under strictly controlled conditions, it was possible to prepare large area R-PE LB film without obvious defect. From the STM image, It could also be seen that R-PE molecules arranged on the mica surface by disc-to-disc to form rod-like structure, which was similar to its self-asembly while adsorbing on HOPG surface. Then, the constituted rod arrayed together to form monolayer. The

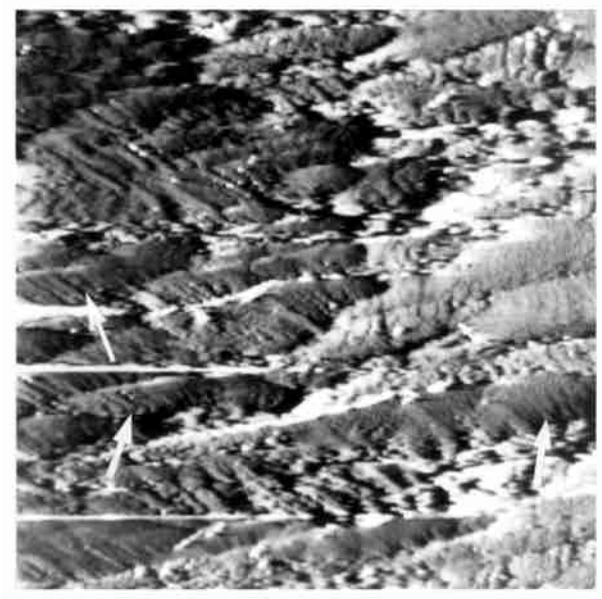

Fig. 5 STM image of R-PE LB film
It = 0.99 nA, Vbias = 458 mV. Scan area: 480 nm × 480 nm.

diameter of the rod was about 50 nm, which was correspond to that of the self assembly on HOPG surface. Every disc in the rod represented one R-PE molecule. Thus, the aggregation form of R-PE in the LB film also verified the rod structure of phycobilisomes *in vivo*. That the size of R-PE moleculaes determined by STM was larger than that by X-ray diffraction was mainly due to the tip-radius-induced artifacts and the effects of gold coating.

Different from all other light-harvesting pigment complexes, which were located within the thylakoid membrane, phycobilisomes were located on the thylakoid surface. The phycobiliprotein discs were assembled by disc-to-disc to form aggregate, which was the most stable state in the cell, and also helpful for the energy transfer. The assembly of phycobiliprotein discs into phycobilisomes is dependent upon the presence of different functional linker polypeptides, and the roles of the linker polypeptides in the aggregate formation and the fine-tuning of the absorption characteristics of phycobiliproteins have been extensively studied[8—11]. Large subcomplexes containing specific linker polypeptides have been isolated after partial dissociation of phycobilisomes[8]. Gottsckalk *et al* (1993, 1994)[10, 11], Glauser *et al* (1993)[9] have reconstituted $(\alpha\beta)_3^{AP}Lc^{8.7}$, $(\alpha\beta)_3^{AP}$ ·21—23 kD [the C-terminal 21—23 kD domain of the core-membrane linker polypeptide L_{CM}], $(\alpha\beta)_3^{PC}L_{RC}^{29.5}(\alpha\beta)_3{}^{AP}Lc^{8.9}$ with purified smaller complexes throughout a complete dissociation of phycobilisomes in low ionic intensity. Since pure phycobiliproteins seem not to be capable of assembly into the structures characteristic of phycobilisomes in solution, it has been inferred that the linker-polypeptides play a key role in the phycobilisome assembly. In this paper, it was found that R-PE molecules could self-assembly together by disc-to-disc while absorbing on HOPG surface, just looked like the rod and core in the phycobilisomes. Therefore, it could be concluded that the formation of the phycobilisome was dependent upon the structure of the phycobiliproteins, and the roles of the linker polypeptides were to locate the phyocobiliproteins in the phycobilisomes and anchor the phycobilisomes to the thylakoid surface, which was because that all the reported phycobiliprotein in bule green algae and red algae had similar disc-like three dimensional structures, otherwise, the linker polypeptides in the rod, core and core-membrane were different.

Phycobiliproteins carry covalently attached linear tetrapyrrole pigments related structurally to biliverdin[1]. The role of phycobiliproteins and phycobilisomes are to absorb light energy and to transfer the energy to reaction centre of photosynthesis where photoinduced charge separation occurs[1]. Due to the stability, high fluorescence yield, large stokes' shifts between absorption and emission, special property of photophysics and photochemistry[15], and the ability of easy preparation for two-dimensional film by LB technique, phycobiliprotein might be acted as a kind of useful materials for crystallization electronics and bioelectronic research.

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海洋红藻多管藻 R-藻红蛋白的体外聚集特性

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摘要 将海洋红藻 R-藻红蛋白(R-PE)吸附到刚揭开的高定向石墨(HOPG)表面上, 然后用扫描隧道显微镜(STM)在纳米尺度上进行直接观察, 发现纯化的 R-藻红蛋白在体外自然聚集时, 能够"面对面"的聚集在一起, 形成非常规则的类似藻胆体的杆状结构。将 R-PE 溶于 2%酒精/水的混合液中, 然后滴加于空气/水界面上, 具有很好的成膜性能。STM 观察结果表明, R-PE 的分子在 Langmuir-Blodgett 膜中的排列方式与其在自然状态下的聚集方式类似, 圆盘状的 R-PE 分子面对面的聚集在一起形成类似藻胆体的杆状结构, 这些"杆"状结构进一步聚集在一起形成膜。以上结果表明, 藻胆蛋白分子在体内以藻胆体的形式存在, 除了连接肽的作用之外, 藻胆蛋白自身的结构特性而导致的分子与分子之间的相互作用, 在藻胆体的组装过程中也起着重要的作用。

关键词 R-藻红蛋白; 体外聚集; 扫描隧道显微镜

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《生命的化学》2002 年第 1 期要目

核糖体基因簇在真菌系统学研究中的意义(赵国柱 张天宇 张 猛) 基因组解析与新药开发(吴晓英 林 影 刘耘) p75 受体信号转导途径的研究进展(毕秀华 袁崇刚) 丙酮酸脱氢酶多酶复合体研究进展(吴永革 尹艳春) 生长激素和生长激素受体的分子生物学(贺淹才) 多酸的细胞渗透作用和抗肿瘤研究(周友亚 李铭岫) PDGF 与疾病的关系(刘 静 金由辛) 转化生长因子-β 对整合蛋白表达与活性的调节(徐 贞 查锡良) 脂质体介导转染法的原理及应用(高 川 王惠芳 张 靖) 蛋白激酶 A 与阿尔茨海默病(孙 莉 刘声远 王建枝)