Investigation of *C*-phycocyanin from blue-green alga *spirulina platensis* with scanning tunneling microscope*

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C-phycocyanin (*C*-PC) was isolated from blue-green alga *spirulina platensis*. A scanning tunneling microscope (STM) has been used to investigate its three-dimensional structure. The samples were dialyzed before the STM experiment, and then deposited on highly oriented pyrolytic graphite (HOPG). The measurement was carried out in ambient condition at room temperature. STM images showed that *C*-phycocyanin was uniformly distributed on solid-state substrate HOPG. The shape of *C*-phycocyanin is disklike with a channel in the center. It is concluded that STM has great potential to observe the structure of biliproteins and phycobilisomes.

I. INTRODUCTION

Phycobilisomes are the light-harvesting antennas of blue-green algas and red algas. They are mainly composed of biliproteins and small amounts of linker polypeptides, which are required for the assembly of the phycobilisomes and their attachment to potosystem II (PSII). There are four major types of biliproteins. They are phycocyanin (PC), phycoerythrin (PE), allophycocyanin (APC), and phycoerythrocyanin (PEC).[1] The energy transfer procedure from quanta absorbed by the peripheral phycobilisome pigment is PE(PEC)→PC→APC→PSII.[1] Hence, structural information of phycobiliproteins and phycobilisomes is very important for understanding the mechanism of light absorption and transfer.

During the last years, the distribution, biochemical composition, and function of *C*-PC have been studied extensively with various analytical tools. Through very hard x-ray crystallography work, simulated crystal structure models of several types of PC have been constructed (one is illustrated in Fig. 1).[2–5] However, until now, there are no results from direct real-space observation of biliiproteins.

Scanning probe microscopies (SPM), particularly, scanning tunneling microscopy (STM) and atomic force microscopy (AFM), have become useful tools for real-space imaging of surfaces. The application of SPM to biomaterials in ambient conditions has growing potential. Many biological objects have been investigated by SPM in the past few years, such as DNA's,[6] DNA-protein complex,[7] polypeptides and protein molecules,[8–12] actins,[13] membranes and membrane proteins,[14–16] etc. In this paper, we first present a series of STM images of *C*-phycocyanin (*C*-PC, one of the major biliproteins of blue-green algas, is composed of two chains, α and β[1]) from *spirulina platensis*.

II. EXPERIMENT

The *C*-PC was purified from blue-green alga, *spirulina platensis*. The biliproteins were extracted by autolysis in distilled water, the *C*-PC was isolated by hydroxylapatite column chromatography, washed with 30 mM phosphate buffer solution (*p*H 6.7, 0.2 M NaCl), the abosorption spectrum was determined with Shimadzu UV-240 spectrophotometer, and the *C*-PC protein concentration was calculated with Roman's method.[1] The sample was dialysed against distilled water for 3 days, 5 μl of diluted *C*-PC solution (approximately 5 μg/ml) was dropped on freshly cleaved HOPG (highly oriented pyrolytic graphite) surface, and then adsorbed on it for 5 min in air at room temperature. The excess solution was removed with filter papers.

STM experiments were carried out in ambient environment with a domestic STM setup CSTM-9000 (manufactured by Institute of Chemistry, Academia Sinica). STM measurement performed with normal STM constant current mode, using tungsten tips made by electrochemical etching. Tunneling current is 0.65 nA, and bias voltage is −260 mV. All STM images presented here are raw data images without any smoothing and filtering.

III. RESULTS AND DISCUSSION

In general, there are some difficulties in the application of STM to biological macromolecules, such as poor conductivity, flexibility and mobility of biomolecules, and effects of salt in the sample solution, etc. These difficulties are major causes of low resolution and lack of reproducibility of STM images. The actual distribution on substrates greatly depends on the concentration and the size of biological objects, and the time allowed for absorption. We have done a lot of experiments to study the characteristics of *C*-PC and salt, in order to avoid the influence of salt, finally, we can control the concentration of the phosphate buffer solution to make *C*-PC molecules distributed uniformly and evenly on graphite (Fig. 2). In our experiment, the sample solution was dialyzed before STM observation, so the effects of salt have been minimized.

Figures 2 and 3 are STM images of *C*-PC molecules on graphite. The pictures clearly show several disk-shaped structures on a graphite surface. Since the samples were dialyzed quite thoroughly before the STM experiments, there

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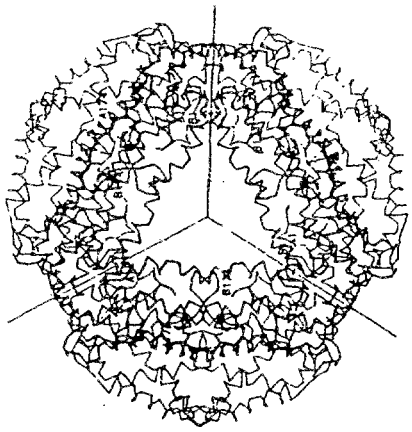

FIG. 1. The *C*-PC hexamer crystal structure model from *agmenellum quadruplicatum* deduced from x-ray crystallography results (Ref. 3). Its diameter is 110 Å, and its thickness is 30 Å.

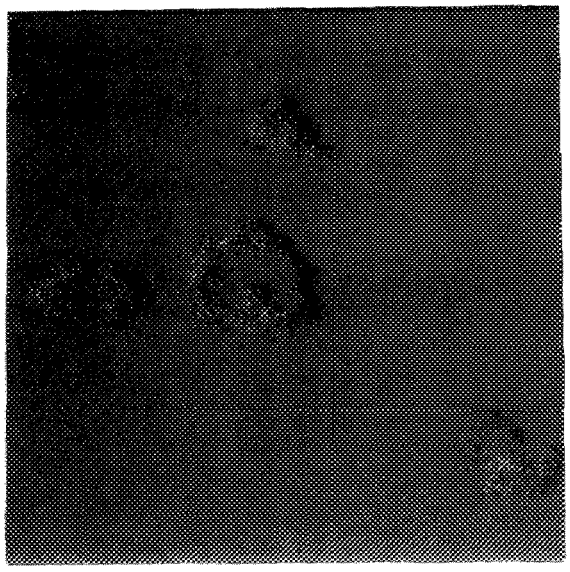

FIG. 3. The STM image of *C*-PC from *spirulina platensis*. V_{bias}=−235 mV, I_t=0.61 nA. Scan area: 200×200 nm^2.

should be little inorganic salt left in the *C*-PC solution, and then the most objects left on the graphite surface should be the *C*-PC molecules. Therefore, we attribute the disklike structures to be the *C*-PC individual molecules. The average lateral size of the disklike shape structures is about 24 nm, although there were some differences in their shape and sizes.

The high resolution STM image of *C*-PC was shown in Fig. 4. The results showed that there was a channel in the center of disk, the diameter of which was around 10–14 nm. The subunits of *C*-PC from *spirulina platensis* are arranged in rings.

The crystal structure models of *C*-PC from *mastigocladus laminosus*,[2] *agmenellum quadruplicatum*,[3] etc. by x-ray crystallography showed that all these reported *C*-PC have similar structures, that is, disklike structures: $(\alpha\beta)_3$ trimers with threefold symmetry and dimension of 110×30 Å,[2] $(\alpha\beta)_6$ hexamer (110×60 Å)^2 by head-to-head association of two trimers. The x-ray crystal structure model resembles the STM images of *C*-PC from *spirulina platensis*, so the x-ray results support our STM observation, and express that we have decreased the salt effects in STM measurements and really obtained the *C*-PC individual molecular structure in real-space. According to the models, the STM image of *C*-PC (Fig. 4) was probably $(\alpha\beta)_3$ or top view of $(\alpha\beta)_6$, every one of the evenly divided three parts of the *C*-PC image might be a monomer: $(\alpha\beta)$.

Compared with the conventional method of x-ray crystallography, the sample preparation for STM is very simple, and the STM now available can process data almost imme-

FIG. 2. The STM image of *C*-PC from *spirulina platensis*. V_{bias}=−235 mV, I_t=0.61 nA. Scan area: 350×350 nm^2.

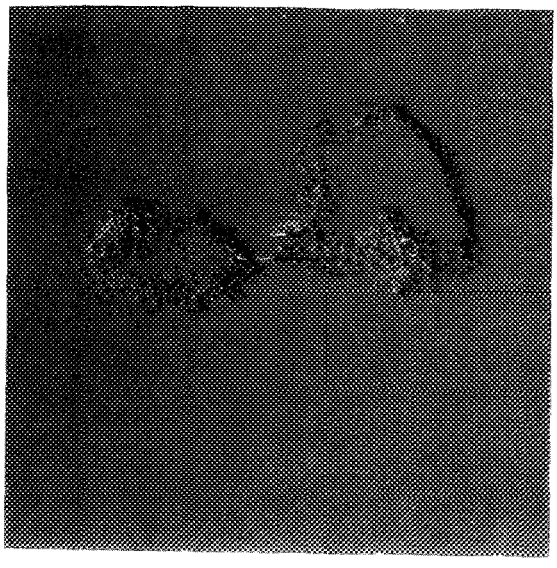

FIG. 4. STM image of *C*-PC from *spirulina platensis*. A high magnification 100×100 nm^2 scan of the same area as Fig. 3, V_{bias}=−235 mV, I_t=0.61 nA.

diately using relatively inexpensive and compact equipment. On the other hand, the result by the x-ray study is deduced from crystals of biological objects, whereas, STM is able to directly investigate the structure of biomaterials under physiological conditions. From the results above, it could be concluded that STM has great potential to study the three-dimensional structure of biliproteins and phycobilisomes. It will help us to directly identify and analyze the structure of photosynthetic apparatus.

IV. CONCLUSIONS

The three-dimensional structure of *C*-PC from *spirulina platensis* was directly observed with a scanning tunneling microscope. It is disk shaped, 240 nm in lateral diameter with a channel in the center, and the results were verified by the *C*-PC crystal structure model deduced from conventional x-ray crystallography. We could conclude that STM is potentially useful to image biliproteins and phycobilisomes.

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