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黄瓜 27 kD LHC- Ⅱ复合物三维结构的电子晶体学初步研究

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摘要: 从黄瓜(*Cucumis sativus* L.)叶片中分离出只含有一种亚基(27 kD)的LHC- Ⅱ复合物。采用 batch 方法获得了其二维晶体,大小为 0.7 μm ×1.0 μm,衍射能力达 30 Å。负染样品的二维投影结果表明,该晶体为 p3 对称性,晶胞参数为 15.4 nm ×15.4 nm,不同于以往报道的菠菜(*Spinacia oleracea* L.)或豌豆(*Pisum satium* L.) LHC- Ⅱ晶体,为另外一种晶型。采用 tomography 技术,收集了 0°~ - 55°系列倾斜照片,进行三维重构。LHC- Ⅱ复合物是由 6 个单体组成的六元环,相邻 2 个单体分别从膜的两侧插膜,方向相反,在膜区靠疏水-疏水相互作用成二聚体,3 个相同的二聚体相互连接成六元环。

关键词: LHC- Ⅱ; 电子晶体学; 三维重构
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Preliminary Study on Electron Crystallography of 27 kD LHC- Ⅱ Complex from Cucumber

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Abstract: Cucumber (*Cucumis sativus* L.) LHC- Ⅱ complex, which consists of only one subunit (27 kD), was isolated and purified. 2-D crystallization was performed by batch method. The crystal is 0.7 μm ×1.0 μm, and diffracts to 30 Å. The projection map of the negatively stained two-dimensional crystal of LHC- Ⅱ complex shows that the crystal has p3 symmetry, lattice constant 15.4 nm ×15.4 nm, which is different from the LHC- Ⅱ of spinach (*Spinacia oleracea* L.) and pea (*Pisum satium* L.). A continuous tomographic tilt series, containing 12 projections from the two-dimensional crystal was subjected to 3-D reconstruction. The 3-D model represents that LHC- Ⅱ complex consists of 6 monomers. These trimer and dimer interactions build up the six-member ring.

Key words: LHC- Ⅱ; electron microscopy; 3-D reconstruction

The light-harvesting chlorophyll a/b complex (LHC- Ⅱ) associated with photosystem Ⅱ in green plants is the most abundant protein complex in the photosynthetic membrane, and alone accounts for roughly half the entire chlorophyll a/b complexes. It collects most of the light energy for transferring to the reaction center, where water is split into H_2 and O_2. LHC- Ⅱ also functions to involve in keeping the photosynthetic membranes stacked and regulate energy distribution between PS Ⅰ and PS Ⅱ.

The structure of LHC- Ⅱ from pea chloroplasts has been determined to 3.4 Å by electron crystallography[1]. This atomic model reveals that LHC- Ⅱ monomer, which binds chlorophyll a (7) and b (5), contains three α-helices and spans the membrane. LHC- Ⅱ is a trimer. Similarly, the 2-D crystal of LHC- Ⅱ complex isolated from spinach is also a trimer studied by electron cryo-microscopy[2]. The structures solved from 2-D crystal from both pea and spinach, however, contains at least two polypeptide isoforms (25 kD and 27 kD). Both polypeptides were suggested to have common, but not identical, structure feature. It is not known whether LHC- Ⅱ trimer consists of one polypeptide or two, but it is most likely that the different isoforms can combine interchangeably into trimer based on high conservity of two isoforms. The biological roles of the isoforms are not fully understood although one isoform is phosphorylated more rapidly than the other, suggesting it may be tightly associated with PS Ⅱ. It is known that phosphorylation of LHC- Ⅱ

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controls energy distribution between PS Ⅰ and PS Ⅱ[3].

In order to understand the structure and function of each isoform of LHC-Ⅱ, the LHC-Ⅱ complexes were isolated and purified from cucumber and spinach. SDS-PAGE showed that LHC-Ⅱ complex from cucumber is dominated by one polypeptide (27 kD) while that from spinach consists of 25 kD and 27 kD isoforms. In this paper, the 2-D crystallization of LHC-Ⅱ from cucumber is presented with the preliminary results of its 3-D reconstruction studied by electron crystallography.

1 Materials and Methods

1.1 Isolation, purification and characterization of LHC-Ⅱ from cucumber and spinach

LHC-Ⅱ was isolated and purified from market spinach (*Spinacia oleracea* L.) and cucumber (*Cucumis sativus* L.) leaves by the method of Lou *et al*[4]. Its emission fluorescence spectra were recorded at 77 K (Hitachi F-4500), the excitation wavelength was 480 nm.

1.2 2-D crystallization of LHC-Ⅱ from cucumber

LHC-Ⅱ was precipitated by adding 4 mol/L KCl (final concentration 300 mmol/L) into 0.5 mL H_2O containing 7.25 μL (7 mg chlorophyll/mL) LHC-Ⅱ and collected by centrifugation. The LHC-Ⅱ, then, was solubilised by 20 μL buffer containing 0.2% Triton X-100, 40% glycerol (W/V), and 10 mmol/L glycine. The solution was incubated for 48 h at 25 ℃ and then heated up to 40 ℃ for 2 h for the formation of 2-D crystals.

1.3 Specimen preparation of electron microscopy

1.5 μL aliquots of samples were applied to freshly prepared and glow-discharged carbon films on 400 mesh copper grids (Plano Inc., Germany). After 1 min, excess solution was blotted off from the side of the grid for six to ten seconds with one layer of filter paper (Whatman No. 1). Specimens were negatively stained with uranyl acetate (2% (W/V)).

1.4 Electron microscopy

Specimens were routinely examined with a Phillips Biotwin 120 electron microscope (Max-Planck-Institute for Biochemistry, Germany) operated at 120 keV and equipped with a Gatan energy filter and a slow-scan CCD camera (1 024 ×1 024). The images were recorded at a magnification of 31 000 and - 500 nm defocus. For 3-D reconstruction, a tilt series of images was taken from nominal 0° to - 55° with an increment of 5°. The total dose for a tilt series was less than 15 e/Å^2. In order to focus the sample for imaging, an area located 2 - 3 μm away from the area of interest was selected for autofocusing. After moving the electron beam back into the area of interest images were taken.

1.5 Image processing

Images were processed on Unix workstation using MPI semper program. Projections were subject to correlation averaging. After first round run, correlation peaks were selected. With the predefined peaks and new reference from the average of odd and even number cell, second round run was carried out, so the unit cell parameters were obtained. Untilted image indicated P6 symmetry. 3-D reconstruction was accomplished by the hybrid Fourier space/real-space approach, starting with unit cells extracted from correlation averages of the tilt series. 3-D models were generated by appropriate threshold by AVS.

2 Results and Discussions

2.1 Characterization of LHC-Ⅱ from cucumber and spinach

LHC-Ⅱ complex was isolated and purified nearly to homogeneity from cucumber and spinach leaves (Fig. 1A). Cucumber LHC-Ⅱ shows mainly one band (27 kD), while two bands (25 kD and 27 kD) for spinach. This result indicated that LHC-Ⅱ complex from cucumber contained one isoform, but spinach LHC-Ⅱ complex consisted of two isoforms. These two LHC-Ⅱ complexes were spectroscopically characterized, they behaved differently. It is known that LHC-Ⅱ binds a minimum of 12 chlorophyll a and b molecules, whose maximum emission wavelengths are 695 nm and 685 nm respectively. As shown in Fig. 1B, chlorophyll a and b from cucumber showed stronger emission of 695 nm than 685 nm, in contrast to spinach LHC-Ⅱ. This result suggests that LHC-Ⅱ complex binds different chlorophyll a and b in cucumber and spinach.

2.2 Image analysis of 2-D crystal of cucumber LHC-Ⅱ

2-D crystal of cucumber LHC-Ⅱ was grown by the batch method[5]. The better crystal for data processing has 0.7 μm ×1.0 μm and appears the arrays examined by electron microscope. The computed Fourier transform of untilted image showed the third order diffraction spots corresponding to 30 Å resolution. Fig. 2 presents the electron micrographic image tilted 50° and its Fourier transform. It clearly shows the (0,3) diffraction spots, especially these spots remain at titled 50°, suggesting that little radiation damage was accumulated during the course of data collection because the low does exposure and auto focus techniques were applied.

A window-filtered image was used as a reference; the crystalline arrays in Fig. 2 containing 1 015 correlation peaks were processed. The projection map calculated without imposing symmetry was strongly suggested to be

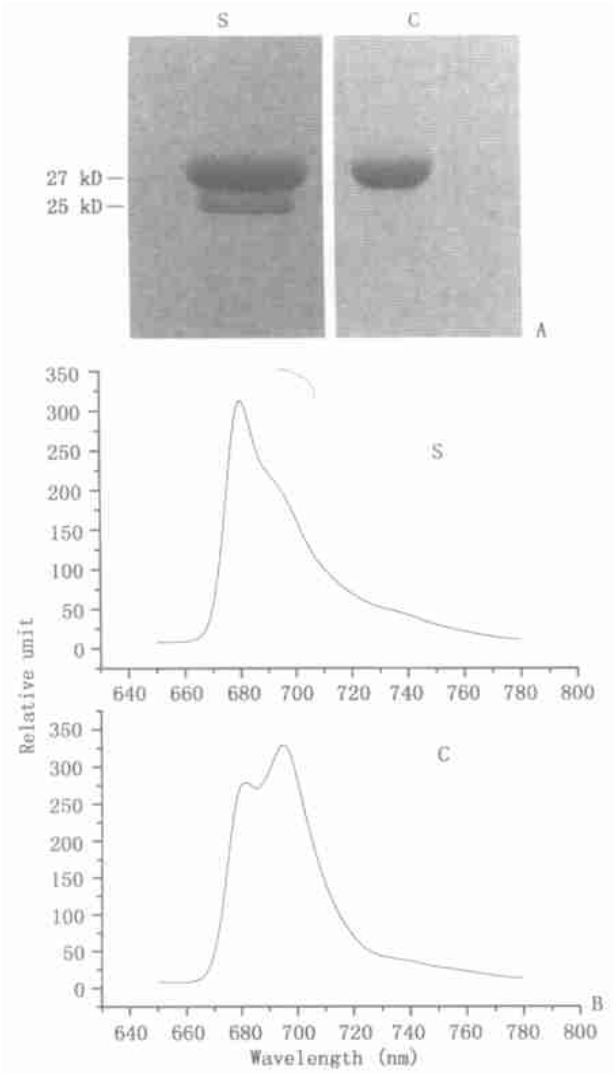

Fig. 1. A. SDS-PAGE of LHC- Ⅱ complex from spinach (S) and cucumber (C). B. Fluoresent emission spectrum of spinach (S) and cucumber (C) at 77 K.

p3 symmetry albeit the computed Fourier transform showed p6 symmetry. By using the average of odd and even peaks as a new reference and predefined correlation peaks, the original image was processed for second round with imposing p3 symmetry, and the root mean square (RMS) deviation values were apparently improved. The 2-D projection map is shown in Fig. 3. The low resolution (～30 Å) map hardly distinguish the arrangement of the subunits, but it is clear that LHC- Ⅱ subunits are associated in a "head-to-tail" fashion around a central cavity, which is consistent with the LHC- Ⅱ of spinach[2]. It was found that the spinach LHC- Ⅱ is a trimer. Its 2-D crystal has p321 symmetry, lattice constant 12.4 nm ×12.4 nm, each unitcell contains two LHC- Ⅱ trimer related by a two-fold axis. Since the resolution of our projection map is low and p6, p321 and p3 have internal correlation, currently it is impossible to exactly assign the symmetry. However, these three kinds of symmetries were applied to calculate the projection map, and no apparent difference was observed at the resolution. Nevertheless, p3 symmetry was applied in the work for safe side due to the lowest symmetry among them. As the lattice constant of this crystal is 15.4 nm ×15.4 nm, which is larger than that of spinach, the crystal we obtained here might appear as a new form.

2.3 3-D reconstruction of cucumber LHC- Ⅱ

A continuous tilt series, containing 12 projections from the 2-D crystal shown in Fig. 2, was subjected to 3-D reconstruction. The actual tilt angles ranged from 1° to - 54°. No significant radiation damage was accumulated while recording the tilt series, as the power spectra of nominal 0° tilts at the beginning and end of the series showed no loss of information.

Each tilted image was processed separately as above and the lattice base vectors as well as the tilt parameters were obtained. Following the 3-D reconstruction procedure established in Max-Planck-Institute for Biochemistry in Germany, 3-D model was generated by appropriate threshold by AVS, as shown in Fig. 4. The threshold for the isosurface representation was set to achieve noise free representation at an estimated protein density of 1.3 g/cm^3.

The model (Fig. 4) shows that, within the unit cell of 15.4 nm lattice constant, there are two sets of protruding trimers on each lattice face. Because the 2-D crystal of LHC- Ⅱ is stabilized by protein-protein interactions, it is reasonable to assume that the 6 prominent surface protrusions correspond to the 6 monomers. Thus, cucumber LHC- Ⅱ complex consists of 6 monomers, which are associated with ring shape. In one unit cell, each asymmetric unit contains a dimer. The adjacent monomers in the opposite orientations form lateral contacts in the hydrophobic interior of the membrane. These trimer and dimer interactions build up the six member ring. In Fig. 4, the thickness of complex is 7 nm, which is consistent with that of the pea LHC- Ⅱ reported as 7.5 nm[6].

It was found that LHC- Ⅱ of spinach or pea is a trimer both *in vivo* and in detergent. Their 2-D crystals have p321 symmetry[7]. As a result of their in-plane 2-fold axis, the crystals can only form if the detergent enables some of the complexes to flip over in the membrane. The detergent, therefore, is very crucial for the crystal formation. However, the cucumber crystal has p3 symmetry, and the adjacent monomers in the opposite orientations form a dimer. This arrangement of subunits has not been observed in the natural membrane. This result suggests that, unlike the LHC- Ⅱ of spinach or pea, the cucumber LHC- Ⅱ in current detergent conditions could be monomers. The monomer could be reconstituted from both

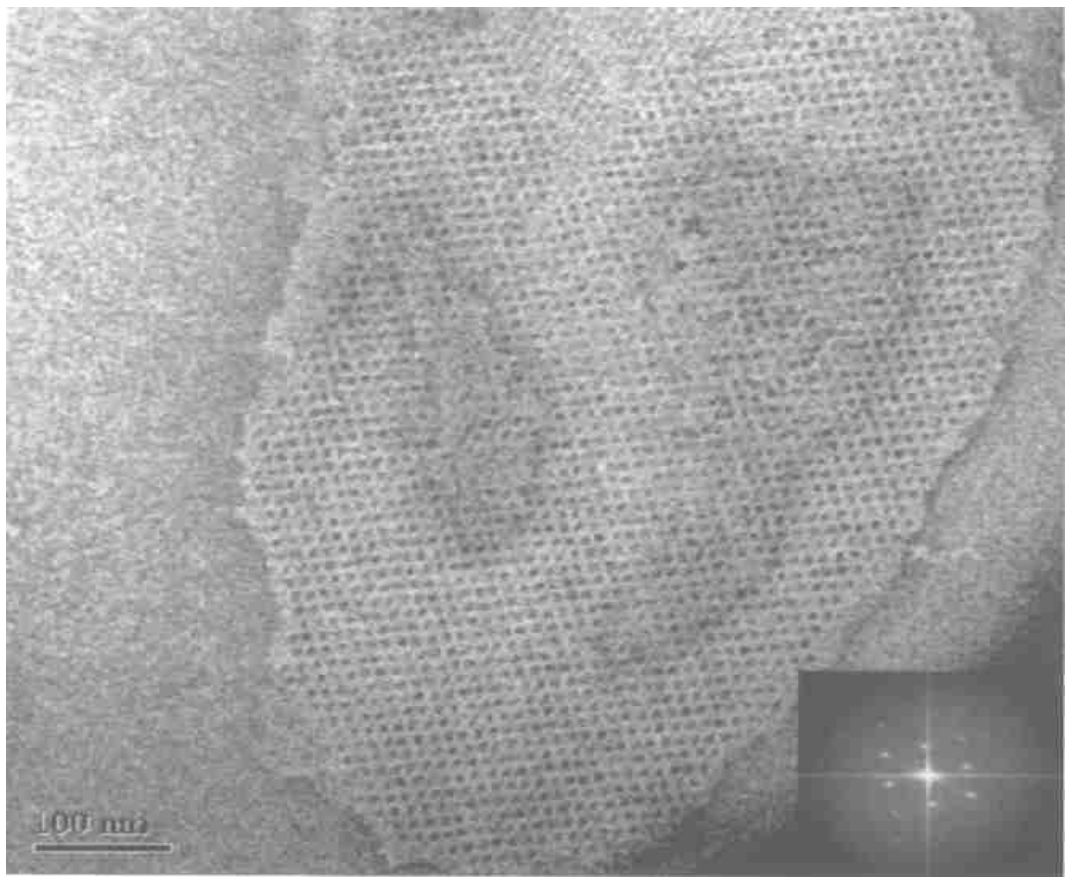

Fig. 2. An electron micrograph of negatively stained 2-D crystal of LHC- II, titled by 50 °from cucumber.

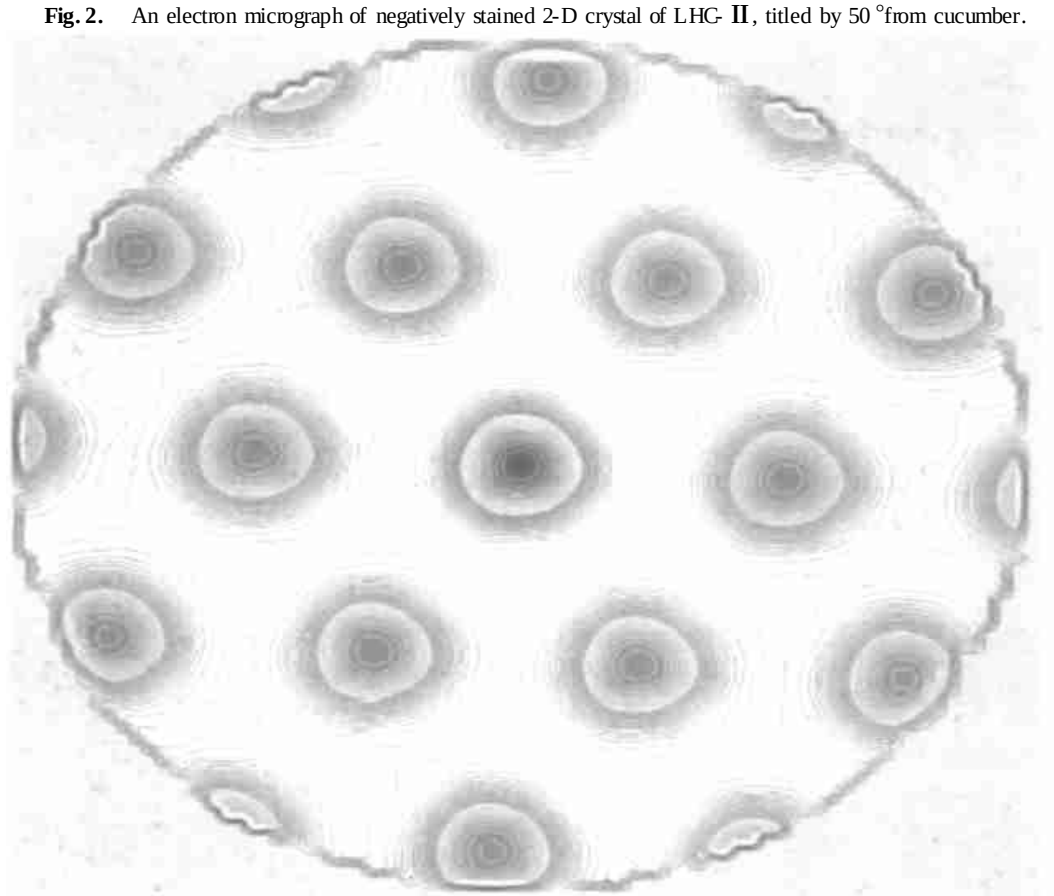

Fig. 3. Projection map of the negatively stained 2-D crystal of LHC- II complex , calculated from the 2-D crystal with p3 symmetry imposed. Correlation average derived from individual motifs.

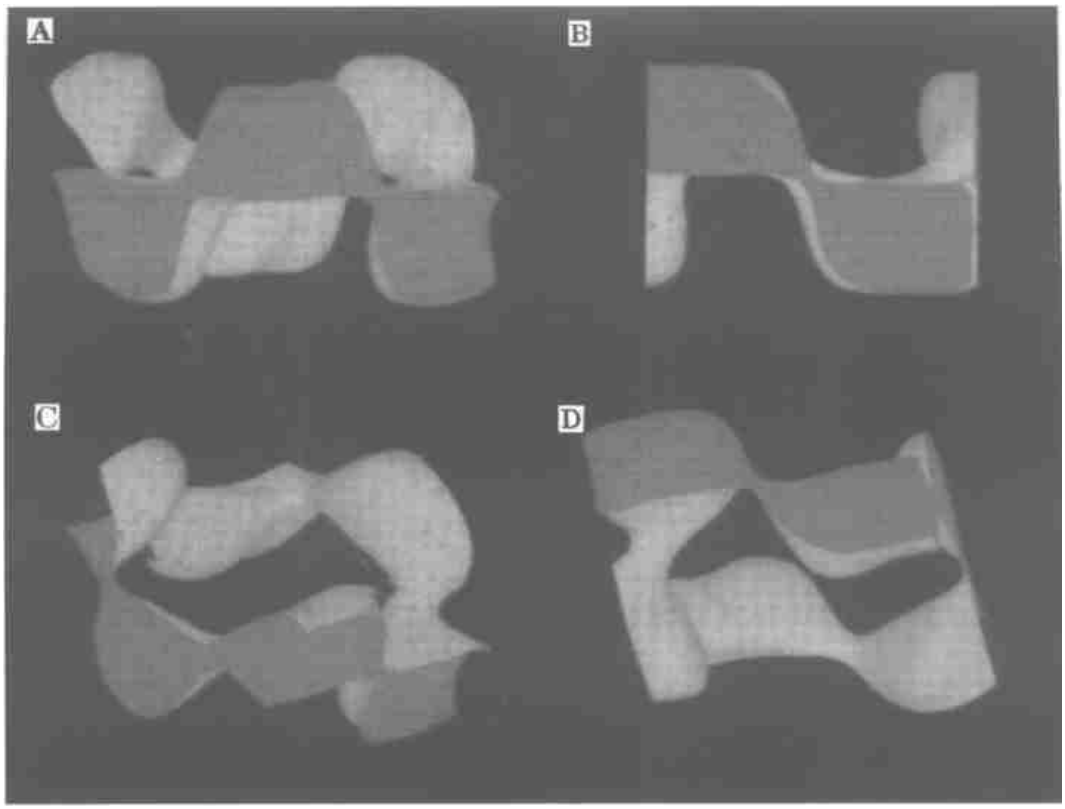

Fig. 4. Three-dimensional reconstruction of LHC-Ⅱ from cucumber. A, B. Side views; C. Top view; D. Bottom view.

sides into the membrane and form two trimers in opposite orientations while LHC-Ⅱ is incubated to form 2-D crystals.

The interaction of lipids with LHC-Ⅱ has an effect on structure and function of LHC-Ⅱ. It is known that DGDG is indispensable for the crystal formation. The obtained crystal is relatively small; it could result from the loss of DGDG to some extent in the course of isolation and purification of LHC-Ⅱ. PG is another phospholipid, which is believed to involve in the trimer formation. It is carrying PG of LHC-Ⅱ monomer that leads to the trimer formation in the natural membrane. However, the trimer can be reversibly dissociated into monomer in the presence of higher concentration of detergent, while PG could still interact with LHC-Ⅱ molecule. Since the 3-D model shows the trimer, implying that PG should exist. Because the monomers are reconstituted from both sides into the membrane, the LHC-Ⅱ could be monomers in the solution due to the higher concentration of Triton X-100. The trimer was formed with the help of PG while the crystal was growing.

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