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**Discovering the mechanism of photosynthesis of an Antarctic alga by infrared light. New clues for extraterrestrial life?**

POINTS:

- Usual plants and algae can only use visible light from the Sun for photosynthesis. An alga grown in the Antarctica can use infrared light for photosynthesis, but the mechanism was not understood.

- By means of cryogenic electron microscopy (cryo-EM), the structure of a protein of the alga which involves in photosynthesis using infrared light has been solved.

- Many planets discovered outside the solar system orbit around fixed stars whose temperature is lower than the Sun and emit infrared light. It has thus been suggested that organisms which utilize infrared for photosynthesis may exist.  The achievements in the present study provide clues for existence of such organisms.

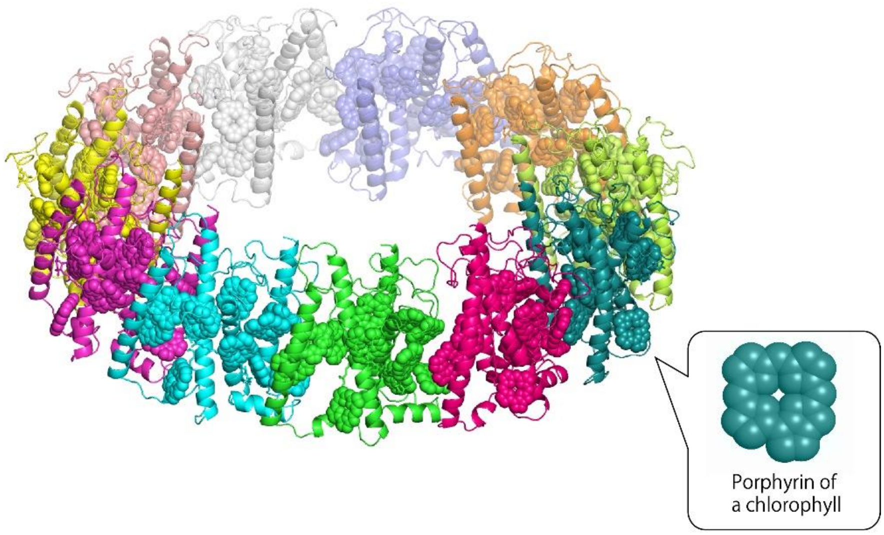


Figure1: Structure of Pc-frLHC shown by 3D model
Each protein subunit is shown by different colors.  Every subunit binds 11 chlorophyll molecules (shown by spheres). The protein part is shown by ribbon model. (credit: Astrobiology Center)

## Summary of the Rsearch:

Research Team consists of following members: Makiko Kosugi, Project Researcher at Astrobiology Center, (Currently Specially Appointed Assistant Professor at National Institute for Basic Biology and Co-researcher at Chuo University); Masato Kawasaki, Associate Professor, Naruhiko Adachi, Specially Appointed Associate Professor, Toshio Moriya, Specially Appointed Associate Professor, and Toshiya Senda, Professor at Structural Biology Research Center, Photon Factory, Institute of Materials Structure Science, High Energy Accelerator Research Organization (KEK); Yutaka Shibata, Associate Professor at Tohoku University; Kojiro Hara, Associate Professor at Akita Prefectural University; Shinichi Takaichi, Former Professor at Tokyo University of Agriculture; Yasuhiro Kamei, RMC Professor at National Institute for Basic Biology; Yasuhiro Kashino, Associate Professor at University of Hyogo; Sakae Kudoh, Professor at National Institute of Polar Research; Hiroyuki Koike, Professor at Chuo University.

They succeeded in to identify Pc-frLHC (*Prasiola crispa* far-red light harvesting chlorophyll binding protein complex) to absorb far-red light (700~800 nm; a part of infrared light) of an Antarctic green alga, *Prasiola crispa*, known to perform oxygenic photosynthesis using far-red light. By means of single particle analysis[*1] by cryogenic electron microscope (cryo-EM) at KEK's facility, 3D structure of the molecule was resolved. Pc-frLHC was found to be a ring-shaped complex with homo undecamer (figure 1). Each subunit binds 11 chlorophyll molecules and 5 out of 11 are suggested to be special chlorophylls involved in the absorption of far-red light.

Spectroscopic analysis indicated that a part of far-red light energy absorbed by special chlorophylls is converted to the same energy level of visible light within the Pc-frLHC and then is utilized for photosynthesis. This work has been published in *Nature Communications* on February 15th, 2023 (Kosugi et al., "Uphill energy transfer mechanism for photosynthesis in an Antarctic alga", 2023).

## Background of the Research：

Plants and algae perform photosynthesis using energy of sun light (350~700 nm) and split water into oxygen, hydrogen and electrons. The reducing power thus obtained is used to assimilate carbon dioxide. The light with wavelength longer than 700 nm is infrared light. It is not used because it does not possess enough energy to split water compared to visible light. Some cyanobacteria[*2] can perform photosynthesis by infrared light and its molecular mechanism has been analyzed, but that of eukaryotic phototrophs[*2] such as plants and algae has not been elucidated.

Recently, the research team led by Makiko Kosugi, Astrobiology Center (Currently at National Institute for Basic Biology), revealed that, *P. crispa*, a eukaryotic photosynthetic organism grown in Antarctica, photosynthesize using far-red light (700~800 nm) which is a part of infrared light and that the efficiency of energy conversion is close to that of visible light (Kosugi et al., 2020). The terrestrial environment of Antarctica is so severe: low temperature, easy to freeze and drought. In addition, the land is exposed to strong ultraviolet light during the summer, which is quite difficult for most of the organisms to survive. *P. crispa* is tolerant to drought and freezing. If it is dehydrated or kept frozen for a long period, the alga can soon revive its metabolic activity just by rewetting with water. *P. crispa* is one of the phototrophs that can survive in the Antarctic terrestrial environment. It forms colonies which are composed of multi-layered sheets of cells (figure 2).

Figure2：Colonies of *Prasiola crispa* formed on rocks around Showa Station in Antarctica (☆ mark on the right earth map). Photo taken during the summer time by the 54th Antarctic Research expedition. The 23cm ruler is placed next to them for comparison. (credit: Astrobiology center)

The cells on surface area of the colony can use visible light for photosynthesis since they can absorb enough amount of light. However, there is also a disadvantage to get damages to the cells by UV light contained in sun light. On the other hand, the cells in the lower layer of the colony have lower possibility to get damages by UV. However, most of visible light for photosynthesis is absorbed by cells on surface area so that the infrared light is dominant in the lower layer.  As the result of long-term adaptation, *P. crispa* has succeeded in using infrared light for photosynthesis. It is assumed that *P. crispa* has propagated by increasing photosynthesis activity at the lower layer of colony by the use of far-red light under the severe environments in Antarctica (figure 3).

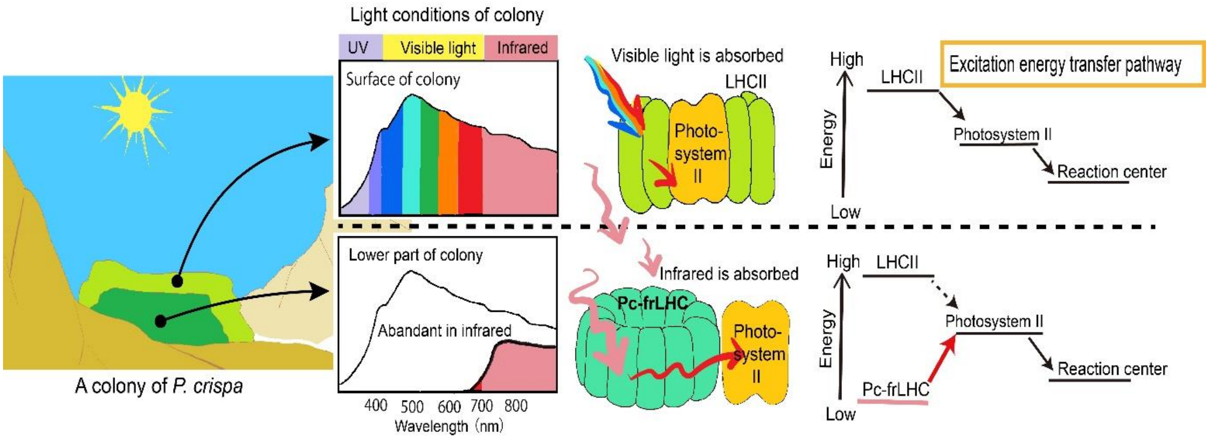


Figure 3: Differences in the light environment and photosynthetic system between surface and lower layer of a colony of *P. crispa* (credit: Astrobiology Center).

The existence of organisms which can use infrared light for photosynthesis is attracting attention in the field of Astrobiology as well. This is because most of exoplanets detected so far are found to orbit around cool stars. Such stars are darker than the Sun and the emitted light contains high proportion of infrared light compared to visible light[*3]. When investigating living organisms in exoplanets, oxygen, which is detectable from the earth, released into the atmosphere by photosynthetic organisms is considered as one of evidences of living organisms. Elucidation of the process of evolution and the mechanism of photosynthesis by infrared light on the earth is important in order to consider the possibility of detection of oxygen at exoplanets orbiting around cool stars. It is also suggested that photosynthesis by infrared light by *P. crispa* contains uphill type energy transfer[*4] process where low energy level molecule will excite high energy level molecule. The mechanism to achieve high efficiency far-red light utilization may include some new quantum biological reaction which has not been recognized so far. They aimed to elucidate the mechanism of far-red light utilizing photosynthesis by purifying the light harvesting protein complex and resolving its molecular structure from *P. crispa*.

## Findings from the Research：

The *P. crispa* used in this research was collected by the 49th and the 54th Japanese Antarctic Research Expedition during their operations. Cells of *P. crispa* were disrupted and the light-harvesting protein complex was separate by the difference of its size and then electrical charge. They purified the protein which has a prominent absorption band in far-red region The protein was named Pc-frLHC (*Prasiola crispa* far-red light harvesting chlorophyll-binding protein complex). Amino acid sequence analysis revealed that Pc-frLHC has a highest homology to that of some green algal LHCI (Light harvesting chlorophyll *a/b* binding complex of photosystem I) of plants and algae. The protein has four transmembrane helices (TMH)[*5] and is bound to photosystem I[*6]. This type of LHCI with 4 TMH is reported to absorb longest wavelength visible light but to absorb far-red light little in a green alga, *Chlamydomonas* (Mozzo et al. 2010). Surprisingly, Pc-frLHC was found to functions as an antenna of photosystem II[*6] which performs water-splitting but not of photosystem I. Thus, it was assumed that absorption band with long wavelength absorbing LHC of the green alga was extended further to longer wavelengths and that the complex functions as a photosystem II antenna.

They succeeded in to collect high resolution data by single particle analysis using cryo-EM and to construct molecular model of Pc-frLHC at atomic resolution. In general, Photosystem II antenna protein complex of green algae consists of three identical subunits. On the other hand, the Pc-frLHC was revealed to be a ring-shaped complex composed of 11 subunits. This type of protein structure has not been reported so far (figure 4). Each subunit binds 11 chlorophylls and all the chlorophylls of the ring complex was found to locate in a position such that distances are close enough to transfer excitation energy and that an energetically connected network is formed. Chlorophyll molecule usually absorbs visible light. However, when some chlorophyll molecules get closer, a part of absorption band shifts to longer wavelengths. In the case of *Chlamydomonas* LHCI with 4 TMH which absorbs longer wavelength light, 2 chlorophyll molecules are located close enough to interact each other (Mozzo et al. 2010). As for Pc-frLHC, it was found that three other chlorophyll molecules are placed close to the 2 chlorophylls and that 5 chlorophylls interact each other. It is assumed that chlorophyll pentamer contributes to far-red light absorption in Pc-frLHC.

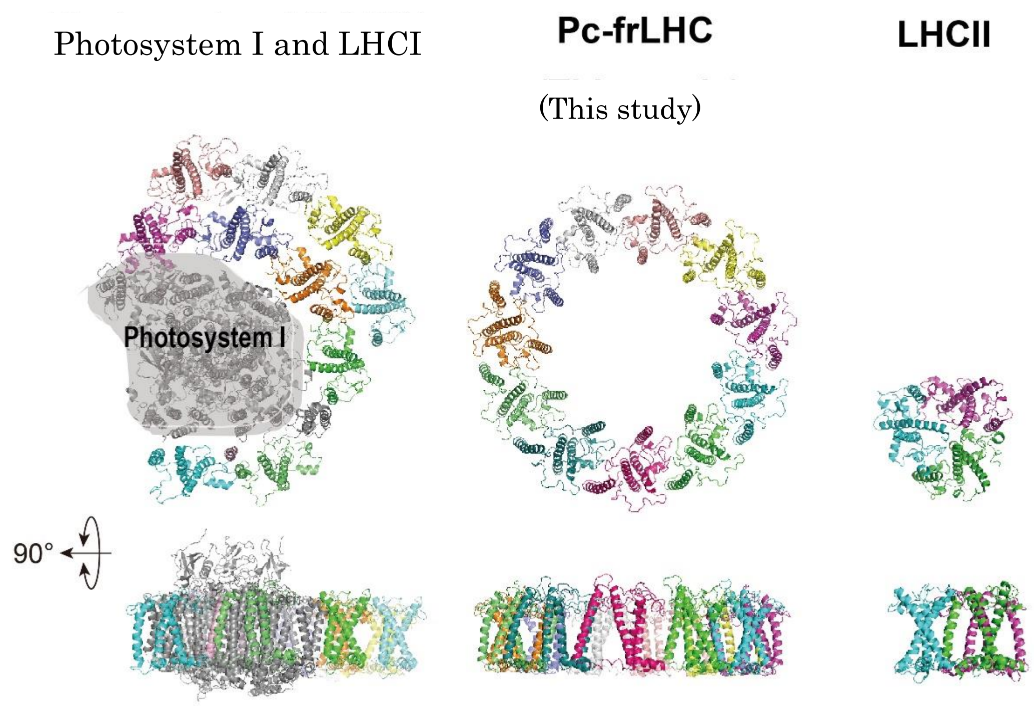


Figure 4 ：Comparison of structures of photosynthetic light-harvesting antenna protein complexes of green algae

Left; Photosystem I core and LHCI bound around it (Protein Data Bank ID: **6jo5**, Suga et al. 2019 *Nature Plants* **5**: 626-636), middle; far-red light harvesting protein complex, discovered in this study and Right; visible-light harvesting protein complex LHCII of photosystem II (Protein Data Bank ID: **1rwt**、Liu et al. 2004. *Nature* **428**: 287-292). (credit: Astrobiology Center)

In order to understand the energy transfer process of far-red light absorbed by Pc-frLHC, they analyzed chlorophyll fluorescence[*7] life time by exciting long wave length form chlorophyll by ultrafast laser. The fluorescence from the long wavelength chlorophyll is emitted at 713 nm, while that from normal chlorophyll is emitted at 680 nm. By analysis of rise kinetics of 680 nm fluorescence, it was revealed that downhill and uphill energy transfer takes place between long wave length chlorophyll and normal chlorophyll within 25 picoseconds (=0.000000000025 seconds). The results indicate that uphill type excitation energy transfer does take place in the Pc-frLHC. It is assumed that, in this process, part of far-red light energy is converted to that of visible light and the subsequent photosynthetic reaction proceeds in the same way as that by visible light.

Future Outlook：

- Elucidate the details of uphill type excitation energy transfer

  In order to fully understand overall mechanism of high efficiency excitation of Photosystem II by far-red light, it is necessary to analyze energy transfer process from Pc-frLHC to photosystem II. Members of the team are planning to purify the super-complexes of Pc-frLHC and phososystem II from *P. crispa* and will elucidate excitation energy transfer process.

- Evolutionary aspects of far-red light utilizing oxygenic photosynthesis

  Although identification or structural analysis of protein complexes are not performed, a number of eukaryotic algae with far-red light absorption band other than *P. crispa* have been reported. It is highly possible that far-red light absorbing light-harvesting protein complex as Pc-frLHC is found in other eukaryotic algae. Analysis of amino acid sequences of light harvesting protein complexes from variety of algae which utilize far-red light for photosynthesis is necessary. The team will clarify their evolutionary aspects and also analyze the similarity and diversity of the mechanism of far-red light using photosynthesis.

- Aspects on Astrobiology

  The search for life on exoplanets is expected to make great progress in the future along with the development of next-generation super-large telescopes. Oxygen is recognized as a key molecule when searching for detectable life evidence (biosignature). Is there any possibility to detect oxygen derived from “photosynthesis” in exoplanets orbiting around cool star? They will search for the possibility of evolution of phototrophs in such exoplanets by elucidating the detailed mechanisms of oxygenic photosynthesis using far-red light on Earth.

Notes

1. Single particle analysis by cryogenic-electron microscopy (Cryo-EM): Rapidly developed technology for structure analysis of proteins. The traditional technique to determine protein structure is X-ray crystallography. It requires crystallization of protein which it is very difficult and fine technique is required. However, it is not necessary in Cryo-EM based analysis. Introduction of cryo-EM makes it easy to analyze protein structure for samples hard to crystallize or hard to prepare large amount such as those from the Antarctica.

2. Cyanobacteria and eukaryotic phototrophs: Cyanobacteria are the most primitive oxygenic photosynthetic organisms and are considered to be the ancestor of chloroplast. Intracellular endosymbiosis of cyanobacteria to non-photosynthetic eukaryotic cells is believed to result in eukaryotic algae and then plants. The mechanism of photosynthesis using far-red light is different between cyanobacteria and eukaryotic phototrophs. It is important to clarify the both mechanisms.

3. Cool star: Also called as M-type dwarf star. It is a fixed star lighter and colder than the Sun (G-type). Since cool stars are predominant fixed stars in the universe, they are considered to be the target for the search for life. As the proportion of infrared light is higher than visible light, the environment of orbiting exoplanets are predominated by infrared.

4. Uphill excitation energy transfer: Excitation-energy transfer between chlorophyll molecules usually takes place from high-level energy molecule to low-level energy molecule. When the reversed reaction occurs, it is called uphill excitation energy transfer. It takes place when energy gap between the molecules is compensated by heat energy.

5. LHC with four transmembrane helices (TMH): Most of light-harvesting antenna protein complexes of algae are folded and embedded in a lipid bilayer called thylakoid membrane in chloroplasts. The number of transmembrane helix depends on that of folding of the protein

6. Photosystem I and Photosystem II: Proteins involved in electron transport in thylakoids of chloroplasts. It contains reaction-center special chlorophyll which induces charge separation by absorbing light energy. Photosystem II splits water. Photosystem I energizes an electron transferred from photosystem II to the level to reduce electron carriers necessary for carbon dioxide fixation. For excitation of photosystem II, higher light energy which corresponds to shorter wavelength than photosystem I, is required.

7. Chlorophyll fluorescence: Light re-emitted when chlorophyll excited by light absorption returns to its non-excited state.

**Research paper Information:**

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