Light-utilizing strategy of cyanobacterium Acaryochloris marina MBIC 11017 based on the bilin chromophore

（課程博士・様式７）（Doctoral qualification by coursework,Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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Title of　Thesis：

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Abstract：

Cyanobacteria originated 2.4 billion years ago as photosynthetic organisms that generate oxygen by utilizing the light. Photosynthesis not only delivers oxygen and energy to the Earth but is also the energy synthesis system for cyanobacteria to survive. Light is the most important energy source for cyanobacteria. They evolved various kinds of light-harvesting systems and light-perception systems. In the case of the light-perception system, cyanobacteria developed bilin-binding phytochromes and cyanobacteriochromes that sense various light qualities covering ultraviolet-to-far-red light regions. In the case of the light-harvesting system, cyanobacteria developed a bilin-binding phycobilisome supercomplex that harvests short wavelength orange light energy and transfers it into the photosystems.

Bilin pigments play important roles for both systems. Phycocyanobilin (PCB) is a main bilin pigment of both light-harvesting and light-perception apparatuses in cyanobacteria. PcyA catalyzes PCB synthesis from biliverdin (BV) via intermediate 18^1, 18^2-dihydrobiliverdin (18^1, 18^2-DHBV). This two-step reaction resulted in stepwise shortening of the conjugated systems and absorbing wavelengths of the pigments.

I focused on the two bilin reductases of unique cyanobacterium *Acaryochloris marina* MBIC 11017 (*A. marina*). *A. marina* exceptionally uses chlorophyll *d* as main photosynthetic pigment that absorbs longer wavelength far-red light than chlorophyll *a*, photosynthetic pigment in most cyanobacteria. In this context, I hypothesized that *A. marina* has photosensory system to sense longer wavelength light than most cyanobacteria. On the other hand, *A. marina* has bilin-binding phycobilisome apparatus to mainly harvest short wavelength orange light, similar to most cyanobacteria. Thus, *A. marina* may sense longer wavelength far-red light and harvest shorter wavelength orange light by using bilin pigments. Interestingly, two PcyA homologs, AmPcyAc and AmPcyAp, were detected from *A. marina* genome, although most cyanobacteria possess only one PcyA. AmPcyAc is encoded on the main chromosome with most photoreceptor genes, whereas

AmPcyAp is encoded on a plasmid with phycobilisome-related genes. This situation suggests that AmPcyAc and AmPcyAp are functionally diversified to supply distinct pigments, 18^1, 18^2-DHBV and PCB, for longer and shorter wavelength light qualities, respectively.

In the present study, I monitored enzyme activities of these enzymes *in vitro*. As a result, 18^1, 18^2-DHBV is highly accumulated for a long period during AmPcyAc enzymatic reaction, whereas 18^1, 18^2-DHBV is transiently accumulated for a very short period during AmPcyAp enzymatic reaction. Furthermore, *in vivo* and *in vitro* reconstitution analyses revealed that the cyanobacteriochrome photoreceptors derived from *A. marina* can incorporate 18^1, 18^2-DHBV and reversibly photoconvert between far-red absorbing form and orange absorbing form, whereas the phycocyanin protein derived from *A. marina* can only incorporate PCB to harvest orange light. These results indicate functional diversification of AmPcyAc and AmPcyAp to provide 18^1, 18^2-DHBV and PCB to the light-perception and light-harvesting systems, respectively. Next, I focused on the residue key for the 18^1, 18^2-DHBV supply to the CBCR photoreceptors by AmPcyAc. Based on the SyPcyA structure, I focused on the 30 residues that form the substrate-binding pocket. Among them, I found that Leu151 and Val225 were both replaced with isoleucine in AmPcyAc. The SyPcyA variant molecule possessing V225I and L151I replacements highly accumulates the 18^1, 18^2-DHBV during the enzymatic reaction and supplies 18^1, 18^2-DHBV to a CBCR derived from *A. marina*. It is of note that the replacement of Val225 with isoleucine was specifically observed in the genus *Acaryochloris*. Taken together, I propose that the specific evolution of PcyA within the *Acaryochloris* genus may correlate with acquisition of Chl. *d* synthetic ability and its growth in longer wavelength far-red light environments.

In this context, I have shown that several CBCRs derived from *A. marina* bind to the 18^1, 18^2-DHBV chromophore and reversibly photoconvert between far-red absorbing form and orange absorbing form. I thus speculate that *A. marina* possesses far-red/orange reversible photoacclimation processes.

Because the original strain of *A. marina* accumulates PBS at very low level, I continuously cultivated this strain under the monochromic orange light condition and obtained the strains adapted to the orange light (OL1, 2, 3, and 4). These strains did not degrade the PBS sufficiently even after being transferred to the far-red light condition, indicating that these strains have been irreversibly adapted to the orange light condition. The resequencing analysis of the OL strains suggested that the copy number of pREB3, which encodes the PBS-related genes, was significantly increased.

In conclusion, the present study has highlighted the physiological importance of 18^1, 18^2-DHBV in *A. marina* and identified the residues of the PcyA enzyme crucial for the 18^1, 18^2-DHBV accumulation. Furthermore, I could obtain *A. marina* strains highly accumulating the PBS, which highlights plasticity of *A. marina* genome in the context of light-harvesting. Taken together, *A. marina* would possess a unique light-utilizing strategy based on the bilin pigment.