光合成細菌を利用した環境保全のための基盤技術開発

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1．研究概要

水素発生，微生物ポリマー，廃棄物処理，環境修復など環境保全のための技術開発は急務の課題である。本研究は光合成細菌の生理生化学，遺伝子工学，生態学などの基礎研究を通じ，光合成細菌を利用した環境保全のための基盤技術の開発を目的とし、次のような研究を行った。

（1）光合成細菌ヘリオバクテリア*Heliobacillus mobilis*からのフェレドキシンの単離と精製に関する研究

（2）褐色を呈するユニークな緑色硫黄細菌*Chlorobium phaeobacteroides*のカロテノイド組成の分析と光環境適応機構に関する研究

（3）緑色硫黄細菌とヘリオバクテリアの鉄硫黄型光化学反応中心周辺の電子伝達経路に関する研究

2．光合成細菌ヘリオバクテリア*Heliobacillus mobilis*からのフェレドキシンの単離と精製に関する研究

PURIFICATION AND CHARACTERIZATION OF TWO FERREDOXINS FROM THE PHOTOSYNTHESTIC BACTERIUM HELIOBACILLUS MOBILIS

Heliobacteria are relatively recently found anoxygenic phototrophic prokaryotes and have bacteriochlorophyll (BChl) g as the major photosynthetic pigment whose chemical structure is rather similar to chlorophyll (Chl) a than to BChl a. The reaction center (RC) of heliobacteria is considered to be similar to that of green sulfur bacteria and PSI of higher plants and cyanobacteria in that they contain very low-potential Fe-S clusters as the secondary electron acceptors. The electron transfer pathway around the RC in heliobacteria remains uncertain. In order to study the electron transfer pathway in heliobacteria from RC to external acceptors, we have purified two ferredoxins (Fd1 and Fd2) from *Heliobacillus mobilis*, and their N-terminal amino acid sequences determined.

They belong to the 2[4Fe-4S] type with absorption maxima at about 280 and 385 nm. Both of them support $NADP^+$ photoreduction in a heterologous assay system containing reaction center particles from the green sulfur bacterium *Chlorobium tepidum* and spinach Fd-

$NADP^+$ reductase. Fd1 was relatively resistant to oxygen and its *A385* value did not significantly decrease in 20 h at 4oC under air, while Fd2 was sensitive to oxygen with its *A385* value reduced by about half in 2 h under the same conditions. We have also sequenced the genes encoding these Fds using a primer-walking strategy. The Fd1 and Fd2 genes are arranged in tandem with an intergenic space of 62 base pairs. The phylogenetic relationship between the Fd1 and Fd2 genes and the relationship of these two genes with other Fds are considered and we concluded that *H. mobolis* Fd genes are the result of gene duplication, although another possibility of horizontal gene transfer of one of the Fd genes can't a priori be ruled out.

3．褐色を呈するユニークな緑色硫黄細菌Chlorobium. phaeobacteroidesのカロテノイド組成の分析と光環境適応機構に関する研究

BROWN SULFUR BACTERIUM *CB.* PHAEOBCTEROIDES CONTROLS CAROTENOID`S COMPOSITION FOR THE PHOTOADAPTATION

Brown sulfur bacterium *Cb. phaeobacteroides* contains various carotenoids (Car) and bacteriochlorophyll (BChl) e in the chlorosomes. But the details of a role and location of Car in chlorosomes have not been clarified yet. In the present study, *Cb. phaeobacteroides* was cultured under various light intensity and the pigments were extracted and analyzed. Many kinds of Car extracted from the cell were identified and they were divided into two groups: the first is Car with one or two f-end groups such as isorenieratene and b-isorenieratene and the second is Car with one or two b-end groups such as b-zeacarotene, b-carotene and trans- and cis-7,8-dihydroxyl-b-carotene. The latter trans- and cis-7,8-dihydro-b-carotene was found to be a novel Car in nature. *Cb. phaeobacteroides* cultured under various light intensities gave the same ratio of Car of the first group to BChl e. However, the ratio of the second Car to BChl e was increased with increasing light intensity.

The differences in absorption spectera were observed for in vitro aggregates of BChl e formed in dimethyl sulfoxide (DMSO)-water solution in the absence and the presence of the two groups of Car. Near 520 nm bands of aggregate of R[E,E]BChl *e* in the presence of Car with f-end groups such as isorenieratene was broadend, but those of R[E,E]BChl *e* with other Car was almost the same as those of R[E,E]BChl *e* without Car. The absorption maximum of Qy bands to that of Soret bands of BChl e ratio (Qy/ Soret) of aggregates of R[E,E]BChl *e* with isorenieratene was the smallest among those of other aggregates.

These results suggest that *Cb. phaeobacteroides* is photoadapted by the change of Car compositions to control the intensity of near 520 nm bands needed to harvest the light energy.

4．緑色硫黄細菌とヘリオバクテリアの鉄硫黄型光化学反応中心周辺の電子伝達経路に関する研究

ELECTRON TRANSPORT PATHWAYS AND KINETICS IN AND AROUND REACTION CENTERS OF GREEN SULFUR BACTERIA AND HELIOBACTERIA

Chlorobium tepidum: A purified RC preparation binds three Fe-S clusters and about one menaquinone per P840. We could not obtain any evidence for its functioning in the main electron transport pathway[5]. We have determined charge recombination rates between oxidized P840 and each of the three reduced clusters by flash-induced absorption spectroscopy[3]. The RC binds two copies of cyt *c*-551, which are kinetically equivalent in electron donation to oxidized P840. The reaction partner of a soluble cyt *c*-554 (about 10 kDa) is bound cyt *c*-551 rather than P840[2]. Our results thus raise the question of to what extent each of cyt bc_1 complex and the soluble cyt *c*-554 contributes to reduction of bound cyt *c*-551 in vivo. Three 2[4Fe-4S] type Fds (more precisely, four isoforms) are present under normal growth conditions. These Fds are efficiently photoreduced by purified RC from the same bacterium[4]. Fd-$NADP^+$ reductase (FNR) of this bacterium is a dimmer in contrast with a higher-plant type FNR that is a monomer[1]. *C. tepidum* FMR shows higher amino acid sequence identity with thioredoxin reductase from some bacteria than with FNR from oxygenic photosynthetic organisms.

Heliobacillus mobilis: We have solubilized and purified RC particles with the detergentTriton X-100, which can reduce $NADP^+$. We have isolated two Fds, one being sensitive and the other rather resistant to oxygen. These Fds can be photoreduced by RC from *C. tepidum*.

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