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Photoadaptation of Green Sulfur Photosynthetic Bacteria *Chlorobium phaeobacteroides*

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Photoadaptation of *Chlorobium* (*Cb.*) *phaeobacteroides* was investigated under dim and strong light intensity. Absorption spectra of these whole cells were different each other: The Soret band intensity and the Qy bandwidth of BChl *e* in cell grown under dim light intensity were smaller and more broadened than those under strong light intensity. From HPLC analysis of the pigments, total carotenoid (Car) / bacterochlorophyll (BChl) *e* ratio of cell increased with increase of light intensities. But composition of BChl *e* homologs almost unchanged. *Cb. phaeobacteroides* contains 11 kinds of Car including isorenieratene and *beta*-isorenieratene as major Car. The compositions of Car were different for cells grown under dim and strong light intensities. In conclusion, *Cb. phaeobacteroides* changes total amount and composition of Car to adapt various light intensities, while homolog composition of BChl *e* unchange.

Key words: photoadaptation, bacteriochlorophyll *e*, *Chlorobium phaeobacteroides*, carotenoids, isorenieratene, *beta*- isorenieratene.

INTRODUCTION

Cb. phaeobacteroides has BChl *e* as an antenna pigment and lives in deep region of lake or pond, while *Cb. tepidum* containing BChl *c* lives near surface of lake. *Cb.* strains have light harvesting apparatus so-called chlorosome, which is covered with a galactolipid monolayer and contains a large amount of BChl and Car and a few amount of protein [1]. BChl *e* differs from BChl *c* in having a formyl group instead of methyl at position 7 such as chlorophyll *b* of plants. BChl might form a rod-like self-aggregate without interaction with protein to harvest light energy. Many kinds of model for self-aggregate of BChl have been proposed [2].

Cb. strains have Car of isorenieratene pathway only. Car biosynthesis pathways are divided into the isorenieratene and chlorobactene pathways. *Cb.tepidum* has chlorobactene and *Cb. phaeobacteroides* has isorenieratene as a major Car, respectively [3]. Total Car / BChl *e* ratio in chlorosome changed by growth conditions. However, Car function in chlorosomes is not well defined yet.

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Cb. phaeobacteroides can grow at -80 m depth of water, in which the light intensity is very weak and the wavelength is virtually limited in the range only from 400 to 600nm. So the antenna apparatus of *Cb. phaeobacteroides* must be constructed to harvest very weak light energy.

In this study, *Cb. phaeobacteroides* was grown under dim and strong light intensities, to investigate how *Cb. phaeobacteroides* adapt under various light intensity efficiently.

MATERIALS AND METHODS

Growth condition. *Cb. phaeobacteriodes* was grown at 30℃ in Pfennig's medium in a 100ml glass test tube under various light intensities of 20W white fluorescence light. The light intensities were adjusted by the distance from the light source and were measured between 400 and 760 nm using the illuminometer. A 10 % culture (the late exponential phase or early stationary phase) was inoculated into freshly prepared medium. The medium was aerated with CO_2 gas to adjust the pH to 6.9. Before the cultures were placed into incubator, kept for 6 hour in dark condition. The bacterial growth was monitored by the absorption at 660nm. Incubation was stopped

at the late exponential phase.

Pigment analysis. The pigments were extracted from whole cells in 10 times volume of methanol-acetone (1:1). BChl *e* homologs, Car / BChl *e* ratio and Car compositions were analyzed by HPLC with a reversed phase column. The identification of Car composition was carried out according to the method described by Takaichi [4].

RESULTS AND DISCUSSION

Cb.phaeobacteroides was grown under dim ($3\,\mu mol \cdot m^{-2} \cdot s^{-1}$) and strong ($48\,\mu mol \cdot m^{-2} \cdot s^{-1}$) light intensities. The absorption maximum of Qy bands of BChl *e* was almost the same but the bandwidth of those (difined as the distance between maxima in a second derivative spectrum) increased from 61 nm for cells grown under strong light intensities to 64.5 nm for those under dim light intensities. When normalized by the Qy bands of those, the Soret bands intensity of BChl *e* of whole cells under strong light intensity was larger than that of cells under dim light intensity (Figure 1).

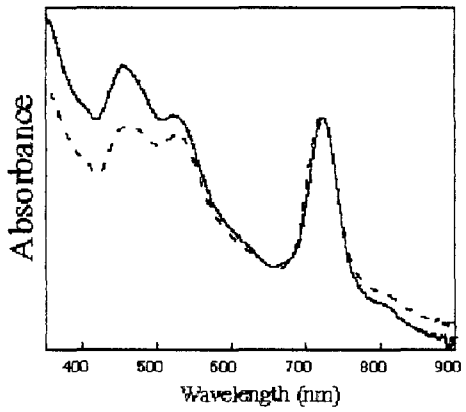

Figure 1 Absorption spectra of cells under dim light and strong light intensities. Normalized by the Qy band of maxima (700 nm) and valley (about 660 nm).

In order to investigate the difference in absorption spectra of whole cells grown under dim and strong light intensities, the pigments were extracted from those and were analyzed by HPLC, respectively. *Cb. phaeobacteroides* contains three major BChl *e* homologs at 8 positions and two epimers at 3^1 positions, R [E, E], R [P, E], S [P, E] and S [I, E] and the minor, S [E, E] and R [I, E], where E, P and I stand for ethyl, propyl and *iso*-butyl groups, respectively [5]. Three major homologs at 8 positions and two epimer at 3^1 positions of BChl *e* ratios of cells grown under dim and strong light intensities were the almost same ratios (data not shown).

Total Car / BChl *e* ratios of cell grown under strong light intensity was about twice that of cell grown under dim light intensity (Table 1). It is known that *Cb.* strain have Car of isorenieratene pathway [2]. Total Car was extracted from cell grown under low and high light intensities and analyzed with reversed phase HPLC (Figure 2): peaks 1, 2, 3, 4, 5 and 6 were identified isorenieratene, *cis*–isorenieratene, chlorobacterene,

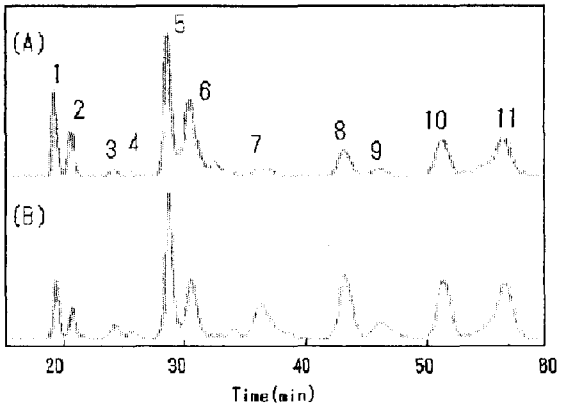

Figure 2. Reversed phase HPLC elution profile of Car extracted from cell grown under dim light (A) and strong light intensities (B).

dihydrochlorobactene, β -isorenieratene and *cis*- β -isorenieratene, respectively, from retention time and absorption spectra of reversed phase HPLC. Other peaks 7-11 might be new Car. *Cb. tepidum* contains exclusively chlorobacterene(3) and dihydrochlorobactene (4) and other peaks 1, 2, 5, 6, 7, 8, 9, 10 and 11 were not detected (data not shown). It is known that *Cb.* strains have Car of isorenieratene pathways.

The Car compositions of cell grown under dim and strong light intensities were different (Figure 2). The major Car as isorenieratene and *beta*-isorenieratene increased in cell grown under dim light intensity but those increasing ratio was different (Table 1).

Table 1. The pigment ratio of cell grown under different light intensities

Light intensity	Car/BChl *e* (A460/A662)	Iso/Car ratio	Biso/Car ratio	Iso/Biso
strong light	0.675	0.078	0.319	0.244
dim light	0.342	0.188	0.507	0.371

The ratio A460/A662 is the sum of peaks dimensions from all Car peak at 460nm divided by the sum of peaks dimensions from all BChl *e* peak at 662nm. Iso shows isorenieratene containing all-trans and cis-isomers. Biso shows *beta*-isorenieratene containing all-trans and cis-isomers.

Cb. tepidum adapted various light intensities: the changes in the ratio of BChl *c* homologs of *Cb. tepidum* were such to give more alkylated as light intensity was low [6]. Additionally, Car composition of *Cb. tepidum* changed also by light intensity. Specifically, the ratio between chlorobactene and dihydrochlorobactene dramatically changed. The maxium of the Qy absorption bands of cells under low light intensity shifted to red and the bandwidth was broadened compared with those of cells under high light intensity. The red sift and broadening of the Qy absorption band makes efficient energy transfer from chlorosome to reaction center.

In these results, absorption spectra of *Cb. phaeobacteroides* might be changed by amount and composition of Car to adapt various light conditions.

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