S1-025

Effects of 3^1R- and 3^1S-epimers of bacteriochlorophyll e on their self-aggregates in chlorosomes of brown-colored green sulfur BACTERIA: an advantage around 520 NM absorption in *Cb. phaeobacteroides*.

H Hirabayashi, T Ishii and K Uehara

Research Institute for Advanced Science and Technology, Osaka Prefecture University, Gakuen-cho, Sakai, Osaka 599-8570, Japan.
Fax: +81-72-254-9935, E-mail: uehara@riast.osakafu-u.ac.jp

Keywords: bacteriochlorophyll *e*, epimer, aggregate, Chlorobium phaeobacteroides, Qx-like absorption band.

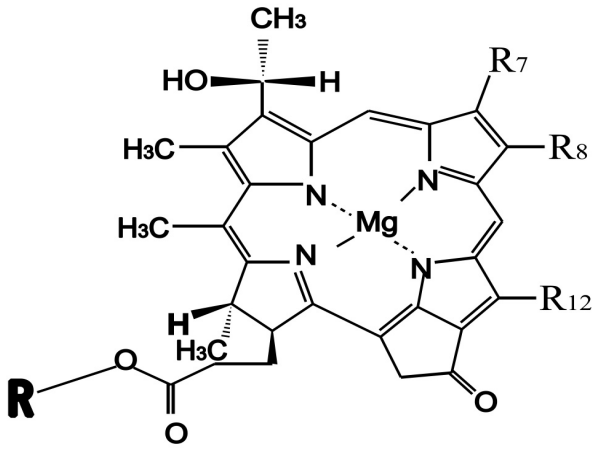
Introduction

Green sulfur bacteria have a unique light-harvesting apparatus so-called chlorosome [1]. Chlorosomes contain a large amount of bacteriochlorophyll (BChl) *c*, *d*, and/or *e* molecules, which self-organize into rod-like supramolecular aggregates together with small amounts of carotenoids and quinones without direct interaction with protein in a lipid monolayer vesicle. *Cb*. *phaeobacteroides* and *Cb. tepidum* contain BChl *e* and BChl *c*, respectively as major chlorosomal BChls. These BChls consist of homologs with different alkyl substituents at positions 8 and 12. Three major homologs of *Cb*. *phaeobacteroides*, [E,E]-, [P,E]-, and [I,E]BChl e_F where E, P and I stand for ethyl, propyl and *iso*-butyl groups, respectively. BChl e has a formyl group at the position 7, which is similar to chlorophyll *b* rather than chlorophyll *a* comparable to BChl *c* (Fig.1). *Cb. tepidum* lives near surface of lake, but *Cb*. *phaeobacteroides* does in the deep region of lake or pond, where the light intensities are very small and the wavelength is limited only from 400nm to 600 nm. The latter grows under a dim light of very narrow wavelength region between 500 and 650 nm [2]. We clarified that *Cb. phaeobacteroides* can live under the dim light with the limited wavelength bands by use of BChl e aggregates absorbing at around 520 nm and increased methylation at the position 8.

Materials and Methods

Cb. phaeobacteoides was grown in batch culture (30 mm diameter of 100 ml Pyrex tubes) at 30°C under various light intensity controlled by changing a distance from a fluorescent lamp. The light intensities were measured between 400 to 760 nm using the illuminometer. Absorbance at 550nm was monitored during the growth of bacteria. The extraction, isolation and purification of BChl e_F and BChl c_F from *Cb. phaeobactroides* and *Cb. tepidum*, respectively were carried out according to the method described by Ishii et al [3]. BChl e_F homologs were separated by HPLC with a reversed phase column.

Artificial aggregates of these BChls were formed in dimethylsulfoxide (DMSO)-water (50:50) that is automatically maintained at pH 7.5 without buffer salts [3]. The aggregation behaviors of the BChls were measured by absorption spectra recorded at 5 min intervals at 30°C. Fluorescence emission spectra of aggregates of BChls were measured at room temperature after aggregation.



	R_7	R_8	R_{12}
BChl *c*	Me	Et/n-Pr/i-Bu	Et
BChl *e*	CHO	Et/n-Pr/i-Bu	Et

Fig.1 Chemical structures of chlorosomal bacteriochlorophyll.

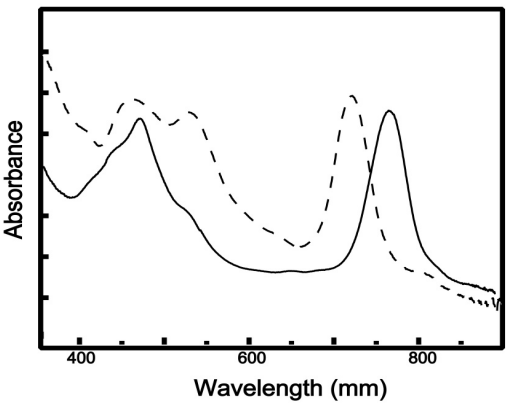


Fig.2 Absorption spectra of *Chlorobium tepidum* containing BChl *c* (solid line) and *Cb.phaeobacteriodes* containing BChl *e* (dashed line).

Results and Discussion

Absorption spectra of living cells for *Cb. phaeobacteroides* and *Cb. tepidum* are shown in Fig. 2. The growth rate and the upper limit of these photosynthetic sulfur bacteria are shown in Fig. 3. The best growths of *Cb. phaeobacteroides* and *Cb.tepidum* were observed under 12μmol m^{-2} s^{-1} and 150μmol m^{-2} s^{-1}, respectively. The growth inhibition by illumination was observed above 48μmol m^{-2} s^{-1} for *Cb. phaeobacteroides*. When *Cb. phaeobacteroides* was grown below 12μ

mol m^{-2} s^{-1}, the growth rate was slower but the upper limit was almost same. The lower light intensities were growth condition of *Cb.phaeobacteroides*, the degree of methylation of BChl *e* at the position 8 increased but total R/S ratio at 3^1-epimer did not change. (Fig.4)

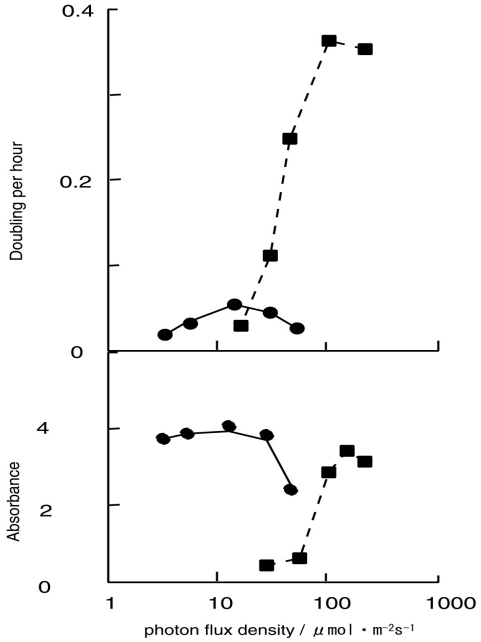


Fig.3 Relationship between the light intensity and the growth rate (doubling per hour in the exponential phase) and the upper limit (measured at 550nm) of *Cb.phaeobacteroides* (—●—) and *Cb.tepidum* (- ■-).(T.Ishii at.el).

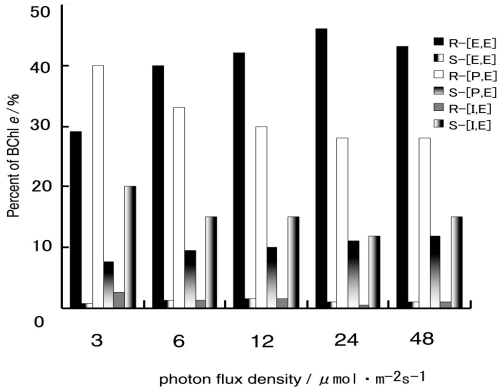


Fig.4 Distribution of BChle homologs in *Cb.phaecobacteroides* grown at different light intensities.

Fig.5 shows the time depended absorption spectra of *in vitro* self-aggregates of BChls in DMSO-water (50:50) at pH 7.5 without buffer solution [3]. The self-aggregates of BChl e_F showed a Qx-like band near 520 nm quite similar to *Cb. phaeobacteroides*, whereas those of BChl c_F did not. Absorption and fluorescence emission spectra of self-aggregates of each BChl e_F homologs were similar to other in vitro system [4, 5] as shown in Table 1. The self-aggregates of R-[P,E] and S-[P,E] BChl e_F showed the different spectral behavior: the Qy bands of absorption spectra for self-aggregates of R-[P,E] and S-[P,E] BChl e_F was different, but fluorescence emission maxima of self-aggregate of R-[P,E] and S-[P,E] BChl e_F was almost the same. The red shift of fluorescence emission maxima increased with increase in the degree of methylation of BChl e_F at the position 8.

Cb. phaeobacteroides can live under a dim light and limited wavelength range because a presence of formyl group at the position 7 of BChl e_F might affect on appearance the absorption band around 520 nm in the BChl aggregates in chlorosome. By the increased methylation at the position 8 of BChl e_F, *Cb. phaeobacteroides* would be adapted to live under more dim light. In

order to establish the structure of the BChl e_F aggregates, FT-Raman spectral studies on the whole cell and in vitro aggregates are now in progress.

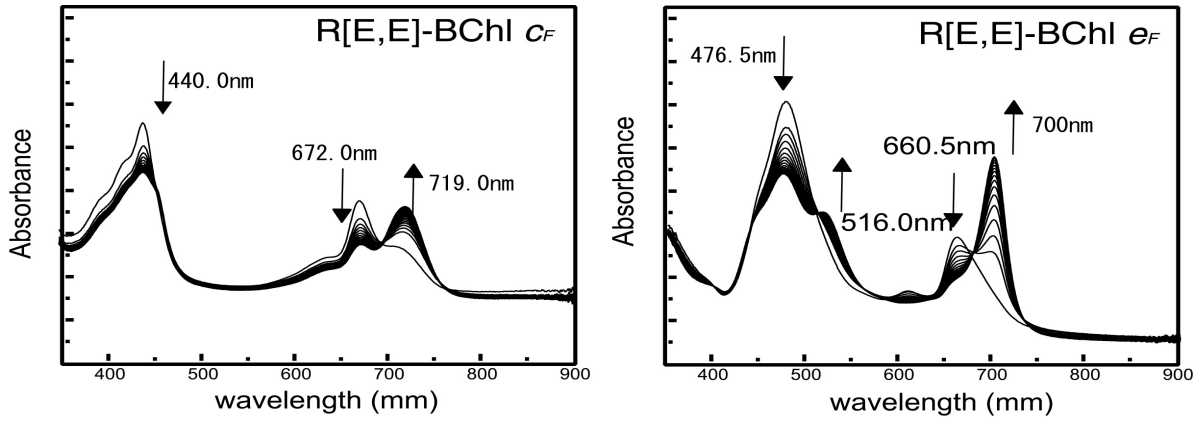


Fig5. Absorption spectra of R-[E,E]BChl *cF* and R-[E,E]BChl *eF* in water-DMSO(50:50)recorded on 5 min interval at 30℃.

Table.1 Optical properties of self-aggregates of BChls in water-DMSO

	Absorption Qy maxima (nm)	Fluorescence emission maxima (nm)
R[E,E]BChl e_F	700	724
R[P,E]BChl e_F	700	729
S[P,E]BChl e_F	720	730
S[I,E]BChl e_F	709	734

Acknowledgements

We thank Prof. LJ Garcia-Gil, University of Girona, Spain and Prof. H Tamiaki, Ritsumeikan University, Japan for providing the culture of *Cb. phaeobacteroides* and also Prof. M Miller, Odense University, Denmark, for supplying the culture of *Cb. tepidum.*

References

[1] JM Olson (1998) *Photochem. Photobiol.*, **67**, 61-75.

[2] CM Borrego, LJ Garcia-Gil, X Vila, XP Cristina, JB Figueras and CA Abella (1999) *FEMS Microbiol. Ecol.* **24**, 301-309.

[3] T Ishii, M Kimura, T Yamamoto, M Kirihata, and K Uehara (2000) *Photochem. Photobiol.*, **71**, 567-573.

[4] DR Steensgaard, H Wackerbarth, P Hildebrandt and AR Holzwarth (2000) *J. Phys. Chem. B*, **104**, 10379-10386.

[5] Y Saga, K Matsuura and H Tamiaki (2001) *Photochem. Photobiol.*, **74**, 72-80.