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The Homologation Process of Bacteriochlorophylls C_F and D_F

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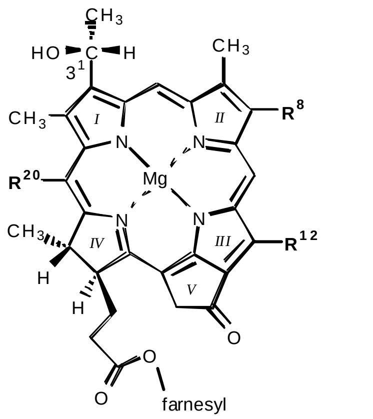
1. Introduction

Green sulfur bacteria such as *Chlorobium limicola* 6230 (DSM 249) (*Cb. limicola*) have characteristic light-harvesting bodies called chlorosomes [1]. The chlorosomes contain bacteriochlorophyll (BChl) *c*, BChl *d* or BChl *e* molecules as light-harvesting antenna pigments exist in rod-like self-aggregates. The chlorosomal BChls of *Cb. limicola* consist of four major homologs possessing different alkyl groups at the positions 8 and 12; *R*-[E,M]-, *R*-[E,E]-, *R*-[P,E]-, and *S*-[I,E]BChl c_F (Fig. 1). The homologation process increases the hydrophobic interactions between the BChl molecules in their aggregates.

With increase in methylation at positions 8, 12, and 20 of the BChls, a concomitant red shift of the absorption spectrum of the chlorosomes has been reported, and it has been proposed that BChl *d* is a precursor of BChl *c*. It is not clear why the BChlsin the chlorosomes of green sulfur bacteria have so many homologs with different alkyl groups.

This study deals with the biosynthesis and the aggregate formation of BChl *c* occurring in *Cb. limicola*.



	R8	R12	R20
BChl *c*	Et/ *n*-Pr/ *i*-Bu	Me/ Et	Me
BChl *d*	Et/ *n*-Pr/ *i*-Bu	Me/ Et	H

Fig. 1 Chemical structures of homologs of BChl *c* and *d*.

2. Materials and Methods

Cb. limicola was grown in batch culture (30 mm diameter of 100 ml Pyrex tubes) at 30 ºC and irradiated at 30 µE/m^2/sec from a fluorescent lamp. Growth medium for *Cb. limicola* was prepared by mixing commercially available inorganic salts and was adjusted to pH 6.9 using CO_2 gas [2]. The concentration of K^+ was about 23 mM for a normal culture.

The ratio of *R*-[P,E]BChl c_F and *R*-[P,E]BChl d_F was calculated from the areas of the HPLC elution bands, and the homologs of the BChls were identified by their absorption and ^{1}H-NMR spectra.

Methyl bacteriochlorophyllide (BChld) (substitution of farnesyl by methyl for the BChl) was synthesized in a 70 % methanol aqueous solution by chlorophyllase which existed in the bacteria. Aggregates of individual BChld homologs formed in dichloromethane- *n*-hexane (90:10) mixture. FT-IR spectra of the BChld aggregates were measured by the microscopic refraction method.

3. Results and discussion

We found that growth under limited potassium concentration (K^+=0.2mM) enriched the BChl *d* homologs coexisting with BChl *c* homologs in chlorosomes of *Cb. Limicola* [3]. A similar effect was observed in the bacterium by addition of 10 mM tetraethylammonium chloride (TEA) which is an inhibitor for passing through the potassium channel. The growth of the bacteria was depressed in the case of less than 0.3 mM K^+ or more than 6 mM TEA and this K^+ effect was not influenced by light intensity. The reversed phase HPLC elution profiles indicate the same proportionality for the four homologs ([E,M]-, [E,E]-, [P,E]- and [I,E]-) of BChl *c* and BChl *d*. However, it is believed that the methylation at positions 8, 12 and 20 proceeds in the cultivation under lower light intensity. The concentration of Na^+, Mg^{2+}, Ca^{2+} and Fe^{2+} influenced only the growth of bacteria, but did not affect the conversion of BChl *d* to BChl *c*. These results suggested that limitation of K^+ ion depresses the biosynthesis (methylation at position 20) of BChl *c* from BChl *d*.

In vitro FT-IR study showed a clear difference in aggregation between BChld *c* and BChld *d*. Aggregates of BChld *d* showed the coordinated 13^1 C=O (1649 to 1653 cm^{-1}) signal which corresponds to the C=O group coordinated to the Mg. On the other hand, BChld *c* showed the strongly hydrogen-bonded together with a coordination of the OH to a Mg atom (Mg･･･OH (3^1)･･･O=C (13^1) linkage; 1643 to 1645 cm^{-1}).

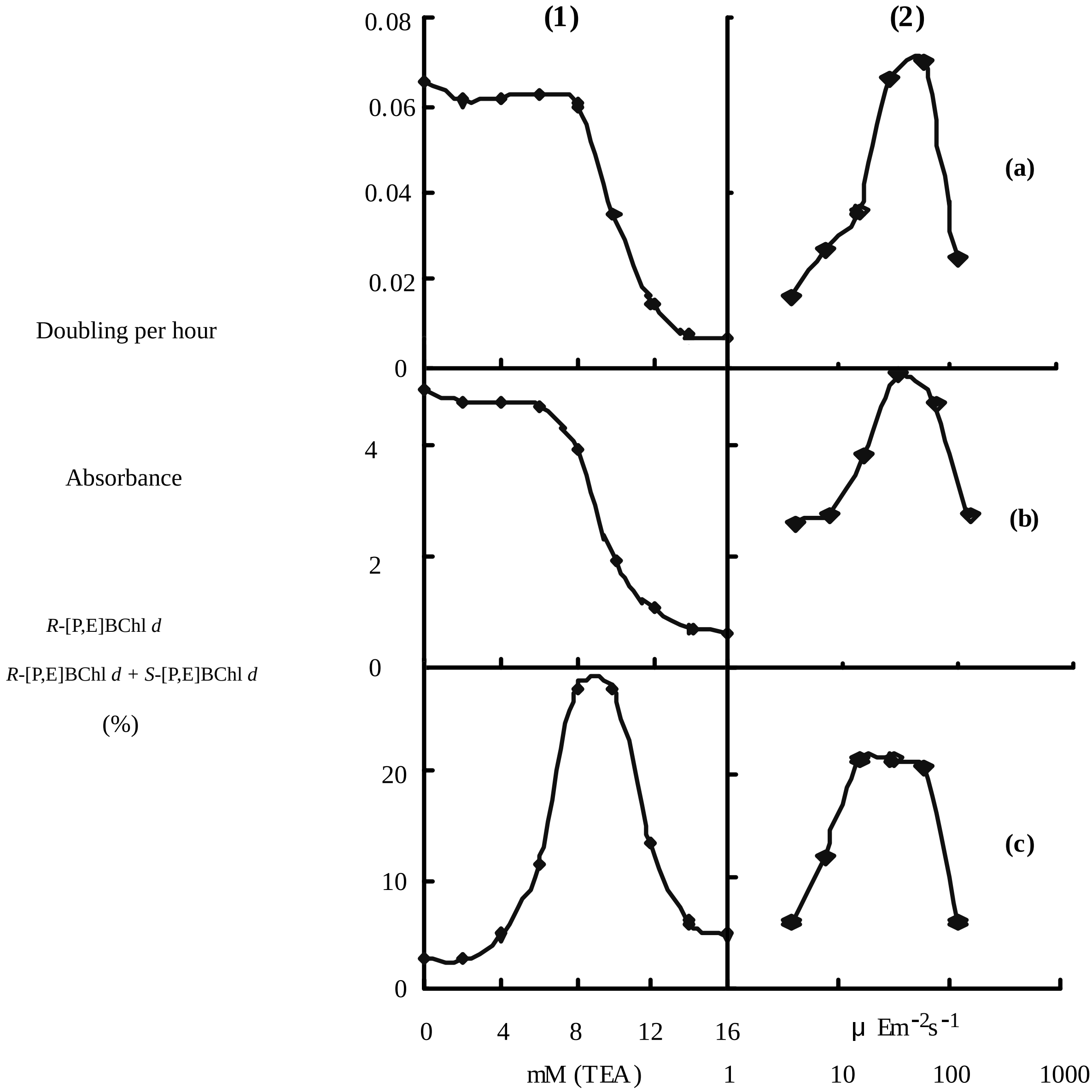


Fig. 3 (1) Relationship between the concentration of tetraethylammonium (TEA) and the growth rate (doubling per hour in the exponential phase)(a), the upper limit (measured at 550 nm)(b) and the ratio of BChl *d* (c). (2) Relationship between the light intensity and the growth rate (doubling per hour in the exponential phase)(a), the upper limit (measured at 550 nm)(b) and the ratio of BChl *d* (c). *Cb. limicola* was cultured under 3.75, 7.5, 15, 30, 60 and 120 μ E/m^2/sec, grown in TEA containing medium (10 mM).

It was observed that the signal of *R*-BChld *c* shifted their coordinated 13^1 C=O bands to lower wavenumber region concomitant with the alkylation at position 8 and 12, but those of BChld *d* showed only a little changes. In the case of *S*-BChld *c*, the signals were observed in higher wavenumber region compared with each homologs of *R* epimer. We proposed that individual homologs form their own structures to create heterogeneity in the rod structures [4]. These results suggest that *Cb*. limicola requires *R*-BChl *c* to form rod-like self-aggregates.

Table. The FT-IR bands (cm^{-1}) of bacteriochlorophyllide (BChld) aggregate.

	Free OH	Coordinated OH	Free C=O	Coordinated keto C=O···Mg	Coordinated keto C=O···H-O···Mg
R-[E,M]BChld d_M		3197		1653	
R-[E,E]BChld d_M		3197		1653	
R-[P,E]BChld d_M	3488 w	3188	1684 w	1652	
R-[I,E]BChld d_M	3479 w	3190	1684 w	1651	
R-[E,E]BChld c_M		3220		1649	
R-[P,E]BChld c_M		3185	1684 w		1645
R-[I,E]BChld c_M	3476 w	3191	1684 w		1643
S-[P,E]BChld c_M	3457	3191	1684 sh	1653	
S-[I,E]BChld c_M	3448 w	3168		1649	

sh, shoulder. w, weak.

References

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