

Effect of Carotenoids on the *in vitro* Aggregation of Bacteriochlorophyll *e*

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In order to investigate effect of the carotenoids (Car) on aggregation of Bacteriochlorophyll (BChl) in chlorosome, we studied the spectral difference in aggregates of BChl *e* formed in the absence and presence of a few kinds of Car in dimethyl sulfoxide (DMSO)–water solution. The absorption spectra of aggregates made of only BChl *e* and those made of a mixture of BChl *e* and Car were almost the same. However, the kinetics and circular dichroism (CD) spectra of aggregate of these were markedly different by kind of Car. Specifically, the rate of aggregation for a mixture of BChl *e* and isorenieratene that contains phenyl as end group was faster than that for only BChl *e*. CD spectra of aggregates made of a mixture of BChl *e* and isorenieratene dramatically changed compared to that made of only BChl *e*. We propose that BChl might form several kinds of rod-like supramolecular structures to in the presence of some kind of Car in chlorosome.

Key words: bacteriochlorophyll *e*, aggregate, carotenoids, isorenieratene, *beta*-carotene, lycopene

INTRODUCTION

Green sulfur bacteria have a unique antenna system called chlorosome. Chlorosome contains a large amount of BChl *c*, *d*, or *e* and Car and a small amount of quinone and protein in a lipid monolayer vesicle. BChls make a rod-like supramolecular structure according to self-aggregate without interaction of protein.

Many reports have described that the absorption spectra of some kind of self-aggregates of BChl *in vitro* is almost same that of chlorosome. In some case, aggregates of BChl *c* *in vitro* give a coordinated bond with $\text{Mg} \cdots \text{OH} (3^1) \cdots \text{O}=\text{C} (13^1)$ based on FT-IR spectral analysis[1]. And the aggregates of BChl *c* in whole living *Chlorobium* (*Cb.*) *limicola* gives also the coordinated bonds with $\text{Mg} \cdots \text{OH} (3^1) \cdots \text{O}=\text{C} (13^1)$ by analysis with near-infrared-FT-Raman, too [2].

Chlorosome contains a large amount of Car, too.

Cb. strains have Car of isorenieratene pathway only in chlorosome [3]. But we cannot understand what role does Car play in chlorosome.

In this study, we investigated that effect of Car influences aggregation of BChl *e* *in vitro*.

MATERIALS AND METHODS

R[E,E]BChl *e* and isorenieratene were extracted from *Cb. phaeobacteroides* and isolated with reverse phase HPLC [4-5]. The aggregations of BChl *e* in the presence of Car (BChl *e*: Car =3:1) were formed in DMSO-water solution (50:50) which is automatically maintained at pH 7.5 without buffer salts. These aggregation behaviors were measured by absorption spectra recorded at 5 min intervals at 30°C. After complete aggregate formation, CD spectra of these BChl *e* aggregates were measured with a JASCO J-720W spectrophotometer.

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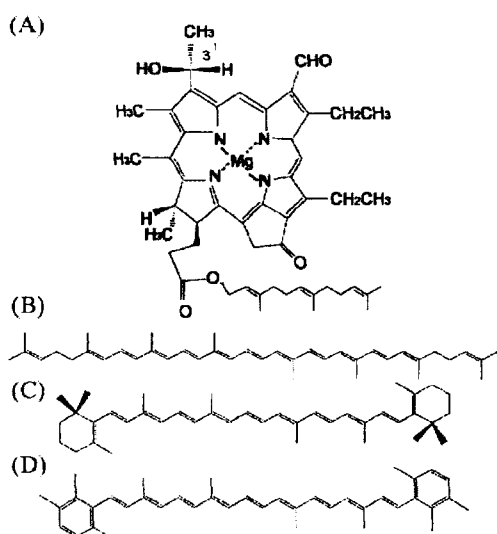


Figure 1 The chemical structure of pigments used. (A) R[E,E] BChl *e*, (B) lycopene, (C) β -carotene, (D) isorenieratene.

RESULTS AND DISCUSSION

In order to investigate the difference in the interaction between BChl *e* and Car, we used three categories of Car: lycopene, β -carotene and isorenieratene (Figure 1). End group of these Cars is liner alkenyl, cyclohexenyl and phenyl, respectively.

The time depended absorption spectra of R [E, E] BChl *e* in the absence and presence of three categories of Car in DMSO-water solution, respectively (Figure 2). It was observed that transformation from monomer (Q_y peak: 660 nm) to aggregates (Q_y peak: 700 nm) with appearance of about 520 nm peak were given for R[E,E] BChl *e*. This absorption spectra is almost the same spectra of whole cell of *Ch. phaeobacteroides* containing BChl *e* as antenna chlorophyll in chlorosome. The absorption spectra of these aggregate were almost the same but the rate of aggregation of R[E,E] BChl *e* was slower than that of mixture of R[E,E] BChl *e* and Car. And the rate of these increased in the order: lycopene (liner alkenyl) < β -carotene (cyclohexenyl) < isorenieratene (phenyl).

Figure 2 shows CD spectra of these aggregate. The CD spectra were different each other depending in kind of

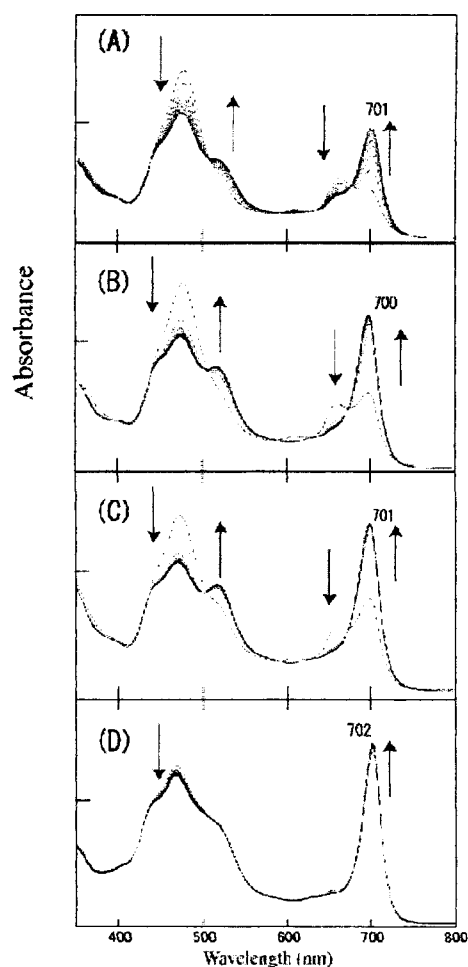


Figure 2 Absorption spectra of the aggregate of only R [E, E] BChl *e* (A), mixture of R [E, E] BChl *e* and lycopene (B), and β -carotene (C), and isorenieratene (D) formed in DMSO-water solution recorded at 5 min intervals, respectively. The arrows indicate the direction of the absorbance changes.

end group of Car. Especially, CD spectra of aggregate of R [E, E] BChl *e* in the presence of isorenieratene is clearly different from that of R [E, E] BChl *e* in the absence of Car. The absorption and CD spectra of the aggregates and kinetics of aggregation of R[E,E]BChl *e* in the presence of benzene instead of isorenieratene which contains end group of phenyl (BChl *e*: benzene = 3 : 0.5) were the same those of R[E,E]BChl *e* without Car (data not shown).

Recently, Car-depleted chlorosome were obtained by means of 2-hydroxybiphenyl-supplemented cultures [6].

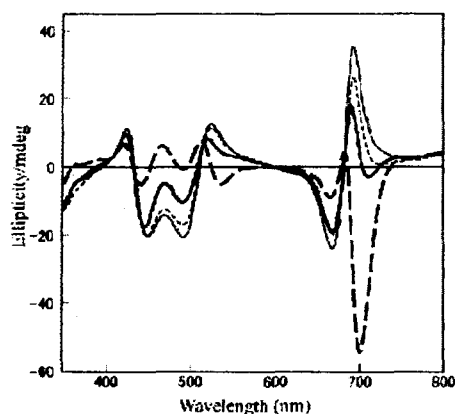


Figure 3 CD spectra of the aggregate of only R[E,E] BChl *e* (solid line), mixture of R[E,E] BChl *e* and lycopene (dashed line), and *beta*-carotene (bold and solid line), and isorenieratene (bold and dashed line) formed in DMSO-water (50:50) solution, respectively.

The CD spectrum of Car-depleted chlorosome was different from that of normal chlorosome.

So, we propose that BChl *e* make various rod-like supramolecular structures to aggregate by interaction with the different category of Car in chlorosome.

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