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Biosynthesis of Unnatural Bacteriochlorophyll *c* Derivatives Esterified with *α,ω*-Diols in the Green Sulfur Photosynthetic Bacterium *Chlorobaculum tepidum*

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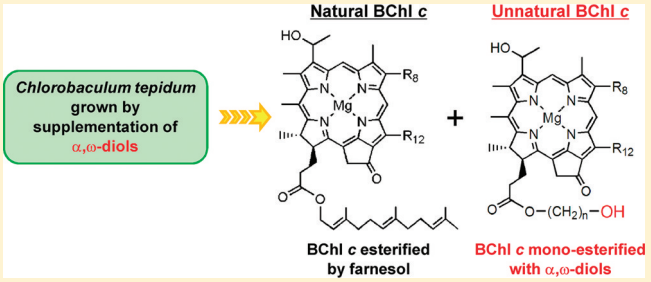
ABSTRACT: Unnatural bacteriochlorophyll (BChl) *c* derivatives possessing a hydroxy group at the terminus of a hydrocarbon chain at the 17-propionate were biosynthesized in the green sulfur photosynthetic bacterium *Chlorobaculum tepidum*. Addition of exogenous 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol in acetone to liquid cultures resulted in accumulation of BChl *c* monoesterified with the corresponding diols. The relative ratios of the novel BChl *c* derivatives esterified with 1,8-, 1,12-, and 1,16-diols to totally producing BChl *c* were 8.2, 50.2, and 57.6% in the cells grown with additive *α,ω*-diols at concentrations of 1.5, 0.06, and 0.06 mM, respectively, at the final concentration. The homologue composition of BChl *c* derivatives esterified with these *α,ω*-diols was similar to that of original, coexisting BChl *c* esterified with farnesol (BChl c_F), suggesting that esterification of *α,ω*-diols occurred at the last step of the BChl *c* biosynthetic pathway by BChl *c* synthase, BchK, in the same manner as in BChl c_F. Chlorosomes, which were isolated from cells grown in the presence of exogenous *α,ω*-diols, contained a ratio and a composition of BChl *c* derivatives esterified with the diols similar to those in the whole cells, indicating that these BChl *c* derivatives were actually present in chlorosomes. Q_y absorption bands of *C. tepidum* cells containing the novel BChl *c* derivatives were shifted to a shorter wavelength, although their bandwidths were analogous to those of cells obtained by normal cultivation. Circular dichroism spectra of cells that had BChl *c* derivatives esterified with *α,ω*-diols exhibited S-shaped signals in the Q_y region, whose polarities were the reverse of those of cells grown in the normal medium and by supplementation with neat acetone as a control experiment. These spectral features of *C. tepidum* possessing BChl *c* derivatives esterified with *α,ω*-diols imply that the novel BChl *c* derivatives possessing a hydroxy group at the terminus of a hydrocarbon chain affect their self-assembly in chlorosomes.



Chlorophyll (Chl) and bacteriochlorophyll (BChl) play important roles in photosynthesis. These photosynthetic pigments capture sunlight energy and convert it into chemical energy in photosynthetic systems with high efficiency. Naturally occurring Chls and BChls have cyclic tetrapyrroles with an exo five-membered ring as the photofunctional moiety and possess a long hydrocarbon chain at the 17-propionate, except for most Chl *c* forms.[1] Esterifying hydrocarbon chains of natural Chls and BChls are not directly conjugated with the photofunctional *π*-macrocycles and do not affect the electronic structures of the cyclic tetrapyrrole moieties. As a result, long hydrocarbon chains of (B)Chl molecules have attracted little attention compared with other peripheral substituents.[2]

BChls *c*, *d*, and *e* are main light-harvesting pigments in green photosynthetic bacteria.[3−6] The molecular structures of BChl *c* in green sulfur photosynthetic bacteria are depicted in Figure 1. BChl *c* molecules in green sulfur bacteria are esterified with farnesol at the 17-propionate. Covalent bonding of a farnesyl chain with the 17-propionate in bacteriochlorophyllide (BChlide) *c* is catalyzed by BChl *c* synthase, BchK, in *Chlorobaculum tepidum* (formerly known as *Chlorobium tepidum*).[7] Additionally, other hydrocarbon chains such as phytol, geranylgeraniol, and hexadecanol have been found in main light-harvesting BChls of green sulfur bacteria.[2,8−13] The green filamentous photosynthetic bacterium *Chloroflexus aurantiacus* mainly possesses BChl *c* esterified with octadecanol, but other minor BChl *c* homologues esterified with phytol, geranylgeraniol, *cis*-9-octadecenol, and hexadecanol are also present in the species.[14] Such varieties in esterifying chains of BChl *c* are in sharp contrast to other (B)Chls, which are esterified with a single long-chain alcohol.[2]

Extramembranous light-harvesting complexes called chlorosomes in green photosynthetic bacteria contain hundreds of thousands of BChl *c*, *d*, and *e* molecules as well as small amounts of proteins.[3−6,15−19] BChl *c*, *d*, and *e* molecules are organized by pigment−pigment interaction and form light-harvesting supramolecular systems. The structures of BChl self-aggregates have been extensively studied, and several

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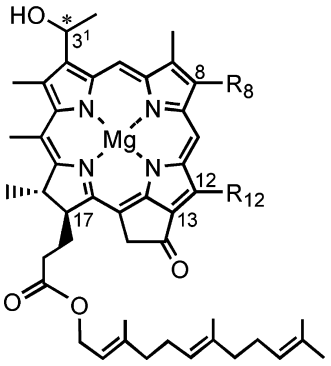

Figure 1. Molecular structures of BChl c_F in *C. tepidum*. R_8 = C_2H_5, *n*-C_3H_7, or *iso*-C_4H_9; R_{12} = CH_3 or C_2H_5.

mesoscopic structural models such as rod-shaped, lamellar, and rolled-up models have been proposed.[20−27] Recent analysis of chlorosomes of a *C. tepidum* mutant, which contained nearly homogeneous BChl *d*, by solid-state nuclear magnetic resonance spectroscopy and cryoelectron microscopy successfully indicated very detailed structural information about BChl self-aggregates.[28] Essential parts of chlorosomal BChl molecules for the self-assemblies are the central magnesium, the 3^1-hydroxy group, and the 13-keto group (see Figure 1). In chlorosomal self-aggregates, the central magnesium of one BChl molecule is coordinated by the 3^1-hydroxy group of another BChl molecule and the hydroxy group is hydrogen-bonded to the 13-keto group of a third BChl molecule.[3−6] Hydrophobic interaction among esterifying hydrocarbon chains in BChl *c* would contribute to formation of chlorosomal self-aggregates. However, effects of these hydrocarbon chains on supramolecular structures of BChl self-assemblies in chlorosomes have not yet been thoroughly unraveled.

In vivo substitution of hydrocarbon chains at the 17-propionate of BChl *c* in green photosynthetic bacteria has been reported.[29−31] Miller and co-workers succeeded in changing the in vivo composition of the esterifying alcohols of BChl *c* in both *C. tepidum* and *Chloroflexus aurantiacus* by cultivating them in liquid cultures containing exogenous alcohols.[29,30] However, little effect on spectral properties of natural chlorosomes was observed: the only detectable change was a slight blue shift (6 nm) of the Q_y band in *C. tepidum* cells, in which 43% BChl *c* was esterified with dodecanol. Mizoguchi and Tamiaki also prepared BChl *c* esterified with octanol and hexadecanol by supplementation with these alcohols through cultivation of *C. tepidum*, but the influence on chlorosomes was not reported.[31]

In vitro preparation of chlorosomal self-assemblies in nonpolar organic solvents and micellar systems allows us to study self-aggregates of metallochlorins esterified with various groups at the 17-propionate. To date, semisynthetic magnesium chlorins and synthetic zinc chlorins, which possess hydrophobic groups,[31−40] hydrophilic groups,[41−44] fluorinated groups,[45−48] and other functional groups,[49,50] have been synthesized, and their self-aggregation has been studied. These reports suggested that interaction of the 17-esterifying chains with each other and with surrounding environments played important roles in supramolecular structures and stabilization of chlorosomal self-assemblies.

In contrast to the in vitro studies of self-assemblies of (semi)synthetic chlorins esterified with various groups, no information is available, to the best of our knowledge, about the introduction of functional groups into esterifying chains at the 17-propionate of natural BChls by biosynthesis in green photosynthetic bacteria to examine effects of such esterifying chains on structures and functions of in vivo chlorosomes. In this study, we make the first report of biosynthesis of BChl *c* esterified with long hydrocarbon chains, whose terminal group is a hydroxy group, in cells of *C. tepidum* by supplementation with exogenous *α,ω*-diols of its liquid culture.

MATERIALS AND METHODS

Apparatus. Visible absorption and circular dichroism (CD) spectra were recorded with a Shimadzu UV-2450 spectrophotometer and a JASCO J-720 spectropolarimeter, respectively. High-performance liquid chromatography (HPLC) was conducted with a Shimadzu LC-20AT pump and an SPD-M20A photodiode array detector. Liquid chromatography−mass spectrometry (LC−MS) analysis was performed with a Shimadzu LCMS-2010EV system equipped with an atmospheric-pressure chemical ionization (APCI) probe. The optical density of cultures was monitored at 660 nm with a Taitec colorimeter (model 518) to estimate growth rates. Ultracentrifugation was performed with a Hitachi CP80MX ultracentrifuge.

Growth Conditions. The liquid medium of *C. tepidum* ATCC 49652 was essentially the same as that in previous reports,[51,52] except for exogenous *α,ω*-diols. 1,8-Octanediol and 1,16-hexadecanediol were purchased from Tokyo Chemical Industry Co., Ltd. 1,12-Dodecanediol was purchased from Wako Pure Chemical Industries, Ltd. *C. tepidum* was grown for 3 days under continuous irradiation with fluorescence lamps (1700 lx) at 43 °C. An acetone solution or suspension containing *α,ω*-diols (1% volume of the culture) was added to the liquid culture every 24 h.

Isolation of Chlorosomes. Harvested cells were disrupted with a Glas-Col BioNeb cell disruption system, and unbroken cells and large debris were removed from the suspension of the broken cells by centrifugation. Chlorosomes were purified by sucrose gradient ultracentrifugation at 220000*g* and 4 °C for 18 h. The main chlorosome band around 15% sucrose-containing buffer was collected and washed three times with 50 mM Tris-HCl buffer (pH 8.0) by ultracentrifugation. Chlorosomes were finally enriched by ultracentrifugation at 280000*g* and 4 °C for 30 min and stored at −78 °C until they were used.

Pigment Analyses. BChl *c* was extracted from harvested cells grown in liquid medium containing *α,ω*-diols and chlorosomes isolated from the harvested cells with an acetone/methanol mixture (1/1, v/v), and the organic suspension was filtered. The organic solution was diluted with diethyl ether, washed with NaCl-saturated water (neutral pH) to remove water-soluble components, and evaporated under reduced pressure. Extracted BChl *c* was dissolved in a small amount of an HPLC eluent and was analyzed on a reverse-phase HPLC column Cosmosil $5C_{18}$-AR-II (6 mm × 250 mm, Nacalai Tesque) with a methanol/water mixture (98/2 or 92/8, v/v) at a flow rate of 1.0 mL/min.

RESULTS

Cultivation of *C. tepidum* by Supplementation with *α,ω*-Diols. *C. tepidum* was grown by supplementation with acetone solutions containing *α,ω*-diols of the liquid medium. Additionally, this bacterium was grown by normal cultivation and by supplementation with neat acetone of the liquid

medium as control experiments. *C. tepidum* grew with a doubling time of 7.6 h in the normal liquid medium under the cultivation conditions described here. In contrast, the doubling time of this bacterium was ~2-fold less by cultivation in the presence of acetone (17.5 h). Figure 2 shows growth curves of

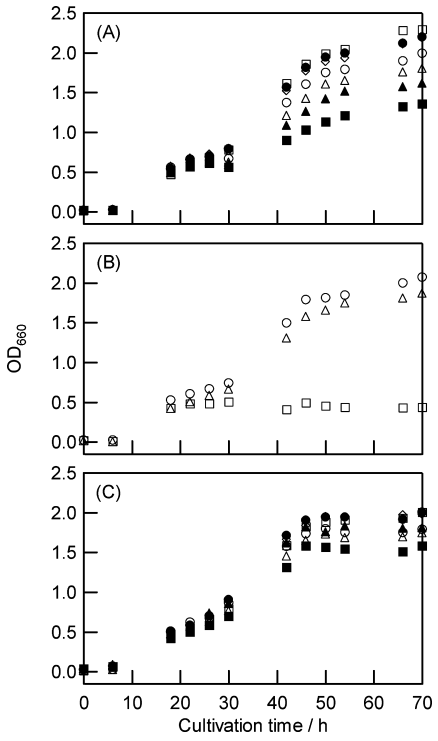

Figure 2. Growth curves of *C. tepidum* by supplementation with 1,8-octanediol (A), 1,12-dodecanediol (B), and 1,16-hexadecanediol (C) at final α,ω-diol concentrations of 0.03 (○), 0.06 (△), 0.15 (□), 0.3 (◇), 0.6 (●), 1.5 (▲), and 3.0 mM (■). Growth curves were obtained by monitoring optical density (OD) at 660 nm.

C. tepidum supplemented with α,ω-diols, and the doubling times of *C. tepidum* grown when supplemented with three α,ω-diols are summarized in Table 1. Addition of an acetone solution containing exogenous 1,8-octanediol and 1,16-hexadecanediol hardly changed the doubling times of *C. tepidum* compared with the cultivation by addition of acetone alone: doubling times of 20.5 and 14.1 h, respectively, with addition of the above acetone solutions, at the final concentration of 1.5 mM. Supplemental 1,12-dodecanediol tended to inhibit the growth of *C. tepidum*: the doubling time was 52.8 h at the final concentration of 0.15 mM, and *C. tepidum* hardly grew in the presence of this substance at higher concentrations.

Analysis of BChl *c* in *C. tepidum*. BChl *c* was extracted from *C. tepidum* cells grown by supplementation with exogenous α,ω-diols and was analyzed by reverse-phase HPLC. Figure 3 depicts representative HPLC elution patterns of BChl *c* from cells grown in the presence of 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol, as well as those by normal cultivation and by supplementation with acetone as a control experiment. *C. tepidum* cells grown in the normal medium and those grown with the addition of acetone contained four major homologues of BChl *c* esterified with farnesol (BChl c_F) as shown in panels A and B of Figure 3, respectively. These BChl c_F homologues were assigned to 8-

Table 1. Summary of Growth Rates and Relative Ratios of BChl *c* Esterified with Supplemental α,ω-Diols to Total BChl *c* of *C. tepidum* Cells by Cultivation with α,ω-Diols

α,ω-diols	final concentration of α,ω-diols in liquid medium (mM)	growth rate (doubling time) (h)	ratio of BChl *c* esterified with α,ω-diols to total BChl *c* (%)
1,8-octanediol	0.03	18.2	not determined
	0.06	21.3	not determined
	0.15	13.9	1.0
	0.3	16.6	2.4
	0.6	15.6	4.2
	1.5	20.5	8.2
	3.0	27.4	12.3
1,12-dodecanediol	0.03	15.3	37.6
	0.06	14.5	50.2
	0.15	52.8	45.4
1,16-hexadecanediol	0.03	15.3	47.0
	0.06	15.1	57.6
	0.15	13.7	54.8
	0.3	13.8	56.0
	0.6	13.8	60.8
	1.5	14.1	56.7
	3.0	14.4	51.1

ethyl-12-methyl ([E,M]), 8,12-diethyl ([E,E]), 8-propyl-12-ethyl ([P,E]), and 8-isobutyl-12-ethyl ([I,E]) BChl c_F in the order of elution by LC−MS measurements and previous data.[51−54] By cultivation with exogenous 1,8-octanediol, new fractions appeared from 5 to 7 min, accompanying the fractions of BChl c_F homologues from 10 to 14 min as shown in Figure 3C. These new fractions exhibited the same visible absorption spectra as BChl c_F homologues, whose absorption bands were positioned at 435 and 670 nm in the HPLC eluent. Thus, the chlorin moieties of these fractions were the same as that of BChl c_F, and their esterifying chains at the 17-propionate were different from the farnesyl chain in conventional BChl c_F homologues. LC−MS analysis of the three new major fractions at 5.7, 6.0, and 6.3 min (OD2, OD3, and OD4, respectively, in Figure 3C) exhibited molecular ion peaks at m/z 731.5, 745.5, and 759.5, respectively. These values were identical to those of their protonated forms (MH^+) of [E,E]-, [P,E]-, and [I,E]-BChl *c* esterified by one hydroxy group of 1,8-octanediol (m/z 731.4, 745.4, and 759.4, respectively). Mass spectra of the fraction at 5.4 min (OD1) could not be measured because the amount of this homologue was too small. The elution pattern of new fractions (OD1−OD4) and visible absorption spectra of fraction OD1 allow us to assign this fraction to [E,M]-BChl *c* esterified by 1,8-octanediol. Therefore, the new BChl *c* fractions in cells grown with 1,8-octanediol were shown to be homologues of BChl *c* esterified by this diol (BChlide *c*-octanediol monoester, denoted BChl c_{OD}).

In the HPLC chromatogram of BChl *c* extracted from cells grown by supplementation with 1,12-dodecanediol (Figure 3D), new fractions, whose absorption spectra were characteristic of BChl *c*-type pigments, appeared from 6 to 9 min. LC−MS analysis of these fractions that eluted at 7.0, 7.4, and 7.9 min (DD2−DD4, respectively, in Figure 3D) showed the molecular ion peaks at m/z 787.5, 801.5, and 815.5, respectively, indicating that these fractions were assigned to

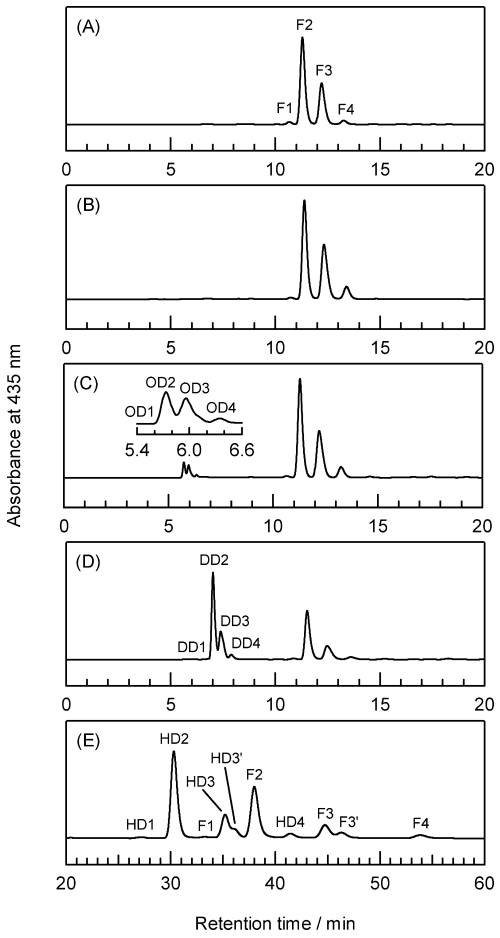

Figure 3. HPLC elution patterns of BChl *c* derivatives extracted from harvested cells of *C. tepidum* grown in a normal medium (A) and by supplementation with acetone (B), 1,8-octanediol (C), 1,12-dodecanediol (D), and 1,16-hexadecanediol (E). The final concentrations of 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol in liquid media were 1.5, 0.06, and 0.06 mM, respectively. The pigments were eluted on a Cosmosil $5C_{18}$-AR-II column at a flow rate of 1.0 mL/min. Mixtures of methanol and water were used as HPLC eluents in chromatograms A–D and E [98/2 and 92/8 (v/v), respectively]. The chromatograms were recorded at 435 nm and normalized at the largest peaks. The fractions of BChl c_F, BChl c_{OD}, BChl c_{DD}, and BChl c_{HD} are denoted by F1–F4, OD1–OD4, DD1–DD4, and HD1–HD4, respectively. The inset in panel C shows a magnified chromatogram of fractions OD1–OD4.

[E,E]-, [P,E]-, and [I,E]-BChl *c*, respectively, esterified by one hydroxy group of 1,12-dodecanediol (BChlide *c*-dodecanediol monoester, denoted BChl c_{DD}). The fraction at 6.8 min (DD1) was assigned to [E,M]-BChl c_{DD} from the elution pattern of new fractions (DD1–DD4) and its visible absorption spectra, although its mass spectra could not be measured because the amounts of this homologue were too small.

Supplementation with 1,16-hexadecanediol of the liquid medium of *C. tepidum* also caused the accumulation of new BChl *c* derivatives as shown in Figure 3E. In this HPLC analysis, the ratio of methanol to water in the HPLC eluent was changed to 92/8 (v/v), because the new BChl *c* derivatives were eluted at a retention time quite similar to that of BChl c_F by using the HPLC eluent in the analysis of BChl c_{OD} and BChl c_{DD} [98/2 (v/v) methanol/water]. Natural BChl c_F homologues eluted at 33.2, 38.0, 44.7, 46.3, and 53.8 min under this HPLC condition. The new fractions at 27.2, 30.3, 35.2, 36.1, and 41.4 min (HD1, HD2, HD3, HD3′, and HD4, respectively, in Figure 3E) exhibited the same visible absorption spectra as the BChl c_F homologues. Their molecular ion peaks in LC–MS analysis were observed at *m/z* 829.6, 843.6, 857.6, 857.6, and 871.7, respectively. These results and the elution patterns reported previously[51−54] allow us to assign the new BChl *c* derivatives to [E,M]-, [E,E]-, 3^1R-[P,E]-, 3^1S-[P,E]-, and [I,E]-BChl *c*, respectively, esterified by one hydroxy group of 1,16-hexadecanediol (BChlide *c*-hexadecanediol monoester, denoted BChl c_{HD}).

The ratios of biosynthesized unnatural BChl *c* derivatives, namely, BChl c_{OD}, BChl c_{DD}, and BChl c_{HD}, to total BChl *c* in *C. tepidum* cells are summarized in Table 1. Small amounts of BChl c_{OD} (1–12%) were biosynthesized in cells grown by supplementation with 1,8-octanediol. In contrast, BChl c_{DD} and BChl c_{HD} accounted for approximately half of the total BChl *c* in *C. tepidum* cells by cultivation with exogenous 1,12-dodecanediol and 1,16-hexadecanediol, respectively.

The homologue composition of BChl c_{OD}, BChl c_{DD}, and BChl c_{HD} in *C. tepidum* cells was estimated from HPLC analysis and was compared with the homologue composition of coexisting BChl c_F. The [E,M]/[E,E]/[P,E]/[I,E] BChl c_{OD} homologue ratio was 0.3/43.8/45.4/10.5 in cells grown at a final 1,8-octanediol concentration of 1.5 mM, which was analogous to the coexisting BChl c_F homologue composition (1.1/56.6/34.6/7.7 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_F). In the case of BChl c_{DD}, the homologue composition of BChl c_{DD} (0.8/64.7/29.3/5.2 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_{DD}) was quite similar to that of BChl c_F (2.0/65.1/26.8/6.1 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_F) in cells that were cultivated in a liquid medium containing 1,12-dodecanediol (final concentration of 0.06 mM). The homologue composition of BChl c_{HD} (1.0/69.2/26.0/3.8 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_{HD}) was also analogous to that of BChl c_F (0.4/66.2/27.8/5.6 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_F) coexisting in cells grown at a final 1,16-hexadecanediol concentration of 0.06 mM. Therefore, all the newly biosynthesized BChl *c* derivatives esterified with *α,ω*-diols had homologue distributions similar to that of BChl c_F, which coexisted in *C. tepidum* cells.

Spectral Properties of *C. tepidum*. Visible absorption spectra of *C. tepidum* cells grown by addition of exogenous *α,ω*-diols are depicted in Figure 4. Cells grown by normal cultivation exhibited Soret and Q_y bands at 461 and 753 nm, respectively (Figure 4A). The main absorption bands of cells when supplemented with acetone as a control experiment were positioned at 459 and 752 nm (Figure 4B), which were similar to those produced by normal cultivation. These indicate that supplementation with acetone during cultivation hardly changes the Soret and Q_y peak positions. In contrast, supplementation with *α,ω*-diols of the liquid culture of *C. tepidum* resulted in the blue shift of Q_y absorption bands of chlorosomes. *C. tepidum* cells grown with 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol exhibited Q_y absorption bands at 747, 737, and 739 nm, respectively. These bands were shifted to shorter wavelengths by 6, 16, and 14 nm, respectively, compared with that of cells grown under normal cultivation. It is worth noting that Q_y absorption bands of monomeric BChl *c* around 670 nm were hardly observed in cells grown in the presence of *α,ω*-diols as shown in Figure 4C–E, indicating that the hydroxy group at the terminus of the hydrocarbon chain in

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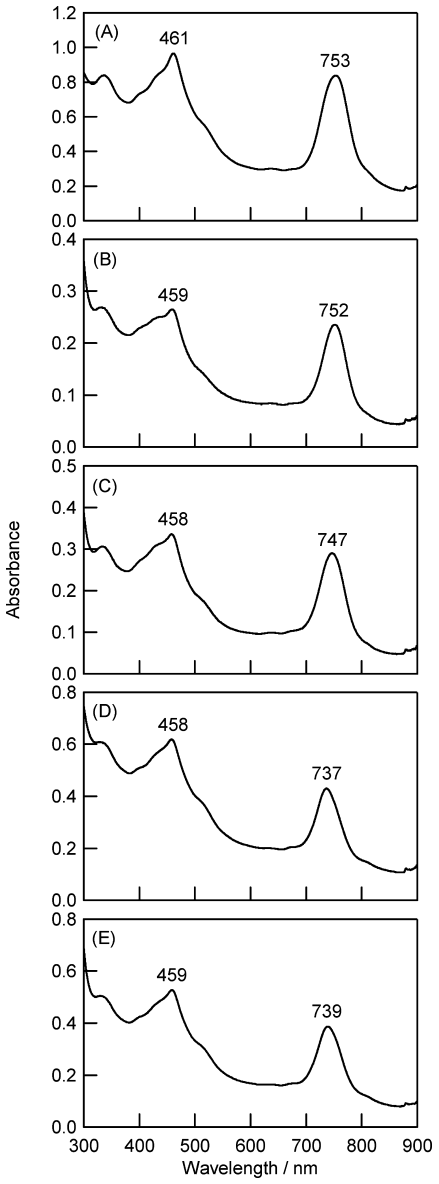

Figure 4. Visible absorption spectra of cells of *C. tepidum* grown in a normal medium (A) and by supplementation with acetone (B), 1,8-octanediol (C), 1,12-dodecanediol (D), and 1,16-hexadecanediol (E). The final concentrations of 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol in liquid media were 1.5, 0.06, and 0.06 mM, respectively. The spectra were recorded after 10-fold dilution of cell cultures with 50 mM Tris-HCl buffer (pH 8.0).

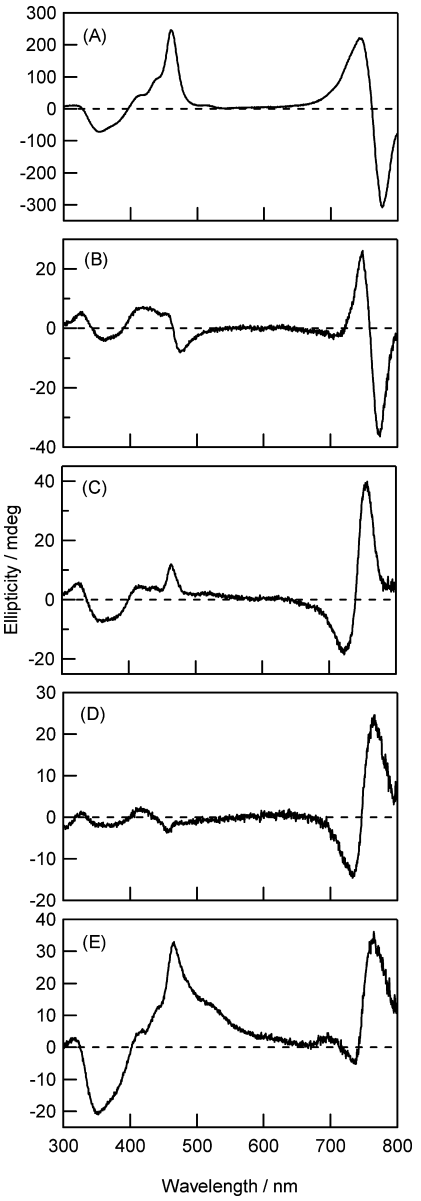

Figure 5. CD spectra of cells of *C. tepidum* grown in a normal medium (A) and by supplementation with acetone (B), 1,8-octanediol (C), 1,12-dodecanediol (D), and 1,16-hexadecanediol (E). The final concentrations of 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol in liquid media were 1.5, 0.06, and 0.06 mM, respectively. The spectra were recorded after 10-fold dilution of cell cultures with 50 mM Tris-HCl buffer (pH 8.0).

the novel BChl *c* derivatives did not prevent in vivo formation of chlorosomal self-assemblies.

Bandwidths, full widths at half-maximum (fwhm), of Q_y absorption bands of *C. tepidum* cells grown with 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol were 961, 945, and 940 cm^{-1}, respectively. These fwhm values were similar to those of cells grown by normal cultivation and by supplementation with neat acetone (1017 and 896 cm^{-1}, respectively).

Figure 5 shows CD spectra of *C. tepidum* cells grown in the presence of exogenous *α,ω*-diols. Cells grown in the normal culture and the acetone-containing culture exhibited reverse S-shaped signals in the Q_y region, as shown in panels A and B of Figure 5, respectively. CD spectroscopy proved that addition of acetone to *C. tepidum* cells under these conditions little changed the polarity of CD signals in the Q_y region. In contrast, cells grown in the presence of 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol had S-shaped signals in panels C–E of Figure 5, respectively, although the Q_y CD signals of cells containing BChl c_{HD} were somewhat different from those of the other cells. Therefore, the CD signals in the Q_y region of *C. tepidum* cells containing BChl *c* derivatives esterified with these *α,ω*-diols were the reverse of the CD signals of cells grown in the normal culture and via supplementation with acetone.

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Analysis of BChl *c* in Isolated Chlorosomes. BChl *c* derivatives were extracted from chlorosomes isolated from the harvested cells grown by supplementation with exogenous 1,12-dodecanediol and 1,16-hexadecanediol and were analyzed by reverse-phase HPLC. BChl *c* derivatives esterified with the supplemental *α*,*ω*-diols, namely, BChl c_{DD} and BChl c_{HD}, were clearly detected as shown in Figure 6. The ratios of BChl c_{DD} and BChl c_{HD} to total BChl *c* in chlorosomes isolated from cells grown in the presence of 1,12-dodecanediol (0.06 mM) and 1,16-hexadecanediol (0.06 mM) were 47.3 and 51.7%, respectively, values quite similar to those in whole cells (see Table 1). The homologue compositions of BChl c_{DD} and BChl c_{HD} in isolated chlorosomes can also be estimated from HPLC analysis. As a result, the homologue compositions of BChl c_{DD} and BChl c_F in isolated chlorosomes were as follows: 1.6/64.5/28.3/5.6 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_{DD} and 2.1/67.4/25.6/4.9 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_F. The homologue compositions of BChl c_{HD} and BChl c_F in isolated chlorosomes were as follows: 1.5/69.6/25.0/3.9 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_{HD} and 0.4/66.5/27.6/5.5 [E,M]/[E,E]/[P,E]/[I,E] for BChl c_F. The homologue ratio of BChl c_{DD} and BChl c_{HD} was analogous to that of BChl c_F coexisting in isolated chlorosomes. This corresponded to the BChl *c* homologue ratio in whole cells described above. These results indicate that the biosynthesized BChl *c* derivatives esterified with *α*,*ω*-diols are actually present in chlorosomes and participate in the formation of self-aggregates.

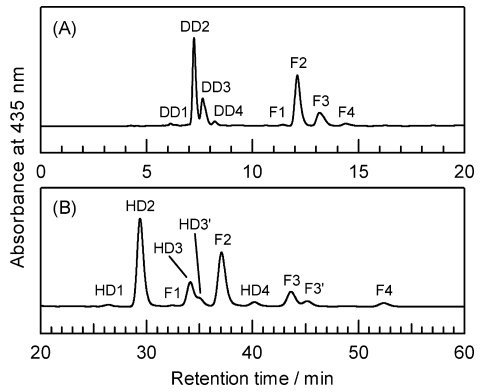

Figure 6. HPLC elution patterns of BChl *c* derivatives in chlorosomes isolated from *C. tepidum* cells grown by supplementation with 1,12-dodecanediol (A) and 1,16-hexadecanediol (B). The final concentrations of 1,12-dodecanediol and 1,16-hexadecanediol in liquid media were 0.06 mM. The pigments were eluted on a Cosmosil 5C_{18}-AR-II column at a flow rate of 1.0 mL/min. Mixtures of methanol and water were used as HPLC eluents for chromatograms A and B [98/2 and 92/8 (v/v), respectively]. The chromatograms were recorded at 435 nm and normalized at the largest peaks. The fractions of BChl c_F, BChl c_{DD}, and BChl c_{HD} are denoted by F1−F4, DD1−DD4, and HD1−HD4, respectively.

DISCUSSION

Biosynthesis of BChl *c* Derivatives Esterified with *α*,*ω*-Diols. This study first demonstrated the biosynthesis of BChl *c* derivatives possessing a hydroxy group at the terminus of a long hydrocarbon chain at the 17-propionate in photosynthetic organisms. The amount of biosynthesized BChl c_{OD} was small, but large amounts of BChl c_{DD} and BChl c_{HD} were synthesized in cells of the green sulfur bacterium *C. tepidum*. A previous report on the biosynthesis of BChl *c* derivatives esterified with supplemental alcohols in *C. tepidum* indicated that the highest ratio of the BChl *c* derivatives to total BChl *c* was 43% when dodecanol was added to liquid media with *β*-cyclodextrin.[30] Hence, our cultivation conditions, including supplementation methods of *α*,*ω*-diols into liquid media, would be appropriate for in vivo attachment of exogenous substrates to BChlide *c* with high efficiency. Additionally, *α*,*ω*-diols could be good substrates on a level similar to that of long-chain monoalcohols for biosynthesis of unnatural BChl *c* derivatives.

The homologue composition of BChl *c* esterified with *α*,*ω*-diols was analogous to that of naturally occurring BChl c_F, which coexisted in *C. tepidum* cells. Such a similarity suggests that attachment of *α*,*ω*-diols to the carboxy group in BChlide *c* was also catalyzed by BChl *c* synthase, BchK, in the last step of the BChl *c* biosynthetic pathway. High ratios of introduction of 1,12-dodecanediol and 1,16-hexadecanediol into BChlide *c* suggest that the terminal hydroxy group, which is not linked to the carboxy group of BChlide *c*, of long *α*,*ω*-diols does not inhibit the enzymatic activity of BchK. In contrast, the low ratio of introduction of 1,8-octanediol into BChlide *c* suggests that short *α*,*ω*-diols might be undesirable substrates for BchK. It was reported that short monoalcohols were rarely connected to BChlide *c* in vivo and were potentially toxic to bacterial growth.[30,31] Therefore, the chain length of alcohols is one of the important factors in appropriate substrates for BchK.

Uptake of *α*,*ω*-diols into *C. tepidum* cells is another possible factor for determining the efficiency of biosynthesis of novel BChl *c* derivatives. Logarithms of the partition coefficients between octanol and water (log P) (which are related to the cell penetrating abilities of chemical compounds) of 1,8-octanediol, 1,12-dodecanediol, and 1,16-hexadecanediol are 0.970, 3.008, and 5.046, respectively (data from SciFinder). These log P values suggest that 1,8-octanediol is probably taken into cells less than the other diols. Such a property of 1,8-octanediol might result in the small amount of biosynthesized BChl c_{OD}.

In this analysis, BChl *c* dimers, in which two BChlide *c* molecules were covalently linked via one molecule of *α*,*ω*-diols, were not detected. This indicates that BChl *c* synthase, BchK, cannot connect another BChlide *c* to the free hydroxy group at the terminus of the long hydrocarbon chain in the novel BChl *c* derivatives. The bulky chlorin moiety would inhibit recognition of the potential substrate, BChlide *c*-diol monoester, in BchK by the steric hindrance.

C. tepidum cells also have BChl *a* and Chl *a* esterified with Δ2,6-phytadienol (Chl a_{PD}) as minor pigments.[2−4,55] Our HPLC conditions did not allow us to analyze the composition and structures of BChl *a* and Chl a_{PD} because the amounts of these pigments are much smaller than the amount of BChl *c*. Further detailed analysis of these minor pigments in *C. tepidum* grown by supplementation with *α*,*ω*-diols will be required.

Effects on Spectral Properties of in Vivo BChl *c* Self-Assembly. Miller and co-workers reported that replacement of farnesol with octadecanol, phytol, and geranylgeraniol as esterifying alcohols of BChl *c* did not change the spectral properties of BChl *c* self-aggregates in *C. tepidum* cells, but esterification with dodecanol shifted the Q_y absorption band to a shorter wavelength by 6 nm.[30] This study indicated that in vivo chlorosomal self-aggregates containing BChl *c* derivatives esterified with *α*,*ω*-diols also exhibited blue-shifted Q_y absorption bands compared with those containing only BChl

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c_F. In contrast, the Q_y bandwidths of chlorosomal self-aggregates containing the novel BChl *c* derivatives were similar to those of aggregates formed by BChl c_F alone. The effects of peripheral substituents in BChl *c* on the spectral properties of in vivo self-aggregates were also reported in *C. tepidum* mutants that were not able to catalyze methylation at the 8^2- and 12^1-positions of BChl *c*; the Q_y peak positions were blue-shifted, and Q_y bandwidths became narrow when the degrees of methylation at the 8^2- and 12^1-positions of BChl *c* decreased.[56] Moreover, *C. tepidum* containing BChl *c* derivatives esterified with *α,ω*-diols showed S-shaped CD signals in the Q_y region, which were quite different from those of cells grown in the normal medium. The CD patterns were retained in chlorosomes isolated from cells grown in the presence of *α,ω*-diols. These results suggest that BChl *c* derivatives esterified with *α,ω*-diols change the supramolecular structures of BChl *c* self-aggregates in chlorosomes, although its nature is unclear. Further studies will be necessary to unravel effects of coexisting unnatural BChl *c* derivatives possessing different esterifying chains on supramolecular structures, spectroscopic properties, and excitation-energy transfer processes of chlorosomes.

In conclusion, exogenous *α,ω*-diols were successfully incorporated into BChl *c* in *C. tepidum* cells. Biosynthesis of the novel BChl *c* derivatives possessing a terminal hydroxy group at the esterifying chain and their participation in photosynthetic light-harvesting complexes will be useful for structural and functional studies of photosynthetic supramolecules as well as development of artificial photosynthetic nanodevices using terminal functional groups of chlorophyllous pigments.

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ABBREVIATIONS

APCI, atmospheric-pressure chemical ionization; BChl, bacteriochlorophyll; BChl c_{DD}, bacteriochlorophyll *c* esterified with 1,12-dodecanediol; BChl c_F, bacteriochlorophyll *c* esterified with farnesol; BChl c_{HD}, bacteriochlorophyll *c* esterified with 1,16-hexadecanediol; BChl c_{OD}, bacteriochlorophyll *c* esterified with 1,8-octanediol; BChlide, bacteriochlorophyllide; CD, circular dichroism; Chl, chlorophyll; Chl a_{PD}, Chl *a* esterified with Δ2,6-phytadienol; [E,E], 8,12-diethyl; [E,M], 8-ethyl-12-methyl; fwhm, full width at half-maximum; HPLC, high-performance liquid chromatography; [I,E], 8-isobutyl-12-ethyl; LC–MS, liquid chromatography–mass spectrometry; [P,E], 8-propyl-12-ethyl.

REFERENCES

(1) Scheer, H. (2006) An overview of chlorophylls and bacteriochlorophylls: Biochemistry, biophysics, functions and applications. In *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications* (Grimm, B., Porra, R. J., Rüdiger, W., and Scheer, H., Eds.) pp 1–26, Springer, Dordrecht, The Netherlands.

(2) Tamiaki, H., Shibata, R., and Mizoguchi, T. (2007) The 17-propionate function of (bacterio)chlorophylls: Biological implication of their long esterifying chains in photosynthetic systems. *Photochem. Photobiol. 83*, 152–162.

(3) Blankenship, R. E., Olson, J. M., and Miller, M. (1995) Antenna complexes from green photosynthetic bacteria. In *Anoxygenic Photosynthetic Bacteria* (Blankenship, R. E., Madigan, M. T., and Bauer, C. E., Eds.) pp 399–435, Kluwer AcademicPublishers, Dordrecht, The Netherlands.

(4) Olson, J. M. (1998) Chlorophyll organization and function in green photosynthetic bacteria. *Photochem. Photobiol. 67*, 61–75.

(5) Tamiaki, H. (2005) Self-aggregates of natural and modified chlorophylls as photosynthetic light-harvesting antenna systems: Substituent effect on the B-ring. *Photochem. Photobiol. Sci. 4*, 675–680.

(6) Miyatake, T., and Tamiaki, H. (2010) Self-aggregates of natural chlorophylls and their synthetic analogues in aqueous media for making light-harvesting systems. *Coord. Chem. Rev. 254*, 2593–2602.

(7) Frigaard, N.-U., Voigt, G. D., and Bryant, D. A. (2002) *Chlorobium tepidum* mutant lacking bacteriochlorophyll *c* made by inactivation of the *bchK* gene, encoding bacteriochlorophyll *c* synthase. *J. Bacteriol. 184*, 3368–3376.

(8) Caple, M. B., Chow, H.-C., and Strouse, C. E. (1978) Photosynthetic pigments of green sulfur bacteria. *J. Biol. Chem. 253*, 6730–6737.

(9) Otte, S. C. M., van de Meent, E. J., van Veelen, P. A., Pundsnes, A. S., and Amesz, J. (1993) Identification of the major chlorosomal bacteriochlorophylls of the green sulfur bacteria *Chlorobium vibrioforme* and *Chlorobium phaeovibrioides*; their function in lateral energy transfer. *Photosynth. Res. 35*, 159–169.

(10) Glaeser, J., Baneras, L., Rütters, H., and Overmann, J. (2002) Novel bacteriochlorophyll *e* structures and species-specific variability of pigment composition in green sulfur bacteria. *Arch. Microbiol. 177*, 475–485.

(11) Airs, R. L., and Keely, B. J. (2003) A high resolution study of the chlorophyll and bacteriochlorophyll pigment distributions in a calcite/gypsum microbial mat. *Org. Geochem. 34*, 539–551.

(12) Massé, A., Airs, R. L., Keely, B. J., and de Wit, R. (2004) The impact of different intensities of green light on the bacteriochlorophyll homologue composition of the *chlorobiaceae Prosthecochloris aestuarii* and *Chlorobium phaeobacteroides*. *Microbiology 150*, 2555–2564.

(13) Manske, A. K., Glaeser, J., Kuypers, M. M. M., and Overmann, J. (2005) Physiology and phylogeny of green sulfur bacteria forming a monospecific phototrophic assemblage at a depth of 100 meters in the black sea. *Appl. Environ. Microbiol. 71*, 8049–8060.

(14) Fages, F., Griebenow, N., Griebenow, K., Holzwarth, A. R., and Schaffner, K. (1990) Characterization of light-harvesting pigments of *Chloroflexus aurantiacus*. Two new chlorophylls: oleyl (octadec-9-enyl) and cetyl (hexadecanyl) bacteriochlorophyllides-*c*. *J. Chem. Soc., Perkin Trans. 1*,, 2791–2797.

(15) Montaño, G. A., Bowen, B. P., LaBelle, J. T., Woodbury, N. W., Pizziconi, V. B., and Blankenship, R. E. (2003) Characterization of *Chlorobium tepidum* chlorosomes: A calculation of bacteriochlorophyll *c* per chlorosomes and oligomer modeling. *Biophys. J. 85*, 2560–2565.

(16) Saga, Y., Shibata, Y., Itoh, S., and Tamiaki, H. (2007) Direct counting of submicrometer-sized photosynthetic apparatus dispersed in medium at cryogenic temperature by confocal laser fluorescence microscopy: Estimation of the number of bacteriochlorophyll *c* in single light-harvesting antenna complexes chlorosomes of green photosynthetic bacteria. *J. Phys. Chem. B 111*, 12605–12609.

(17) Niedermeier, G., Scheer, H., and Feick, R. G. (1992) The functional role of protein in the organization of bacteriochlorophyll *c* in chlorosomes of *Chloroflexus aurantiacus*. *Eur. J. Biochem. 204*, 685–692.

(18) Vassilieva, E. V., Stirewalt, V. L., Jakobs, C. U., Frigaard, N.-U., Inoue-Sakamoto, K., Baker, M. A., Sotak, A., and Bryant, D. A. (2002) Subcellular localization of chlorosome proteins in *Chlorobium tepidum*

and characterization of three new chlorosome proteins: CsmF, CsmH, and CsmX. *Biochemistry 41*, 4358−4370.

(19) Li, H., and Bryant, D. A. (2009) Envelope proteins of the CsmB/CsmF and CsmC/CsmD motif families influence the size, shape, and composition of chlorosomes in *Chlorobaculum tepidum*. *J. Bacteriol. 191*, 7109−7120.

(20) Staehelin, L. A., Golecki, J. R., Fuller, R. C., and Drews, G. (1978) Visualization of the supramolecular architecture of chlorosomes (*Chlorobium* type vesicles) in freeze-fractured cells of *Chloroflexus aurantiacus*. *Arch. Microbiol. 119*, 269−277.

(21) Staehelin, L. A., Golecki, J. R., and Drews, G. (1980) Supramolecular organization of chlorosomes (*Chlorobium* vesicles) and of their membrane attachment sites in *Chlorobium limicola*. *Biochim. Biophys. Acta 589*, 30−45.

(22) Saga, Y., and Tamiaki, H. (2006) Transmission electron microscopic study on supramolecular nanostructures of bacteriochlorophyll self-aggregates in chlorosomes of green photosynthetic bacteria. *J. Biosci. Bioeng. 102*, 118−123.

(23) Egawa, A., Fujiwara, T., Mizoguchi, T., Kakitani, Y., Koyama, Y., and Akutsu, H. (2007) Structure of the light-harvesting bacteriochlorophyll *c* assembly in chlorosomes from *Chlorobium limicola* determined by solid-state NMR. *Proc. Natl. Acad. Sci. U.S.A. 104*, 790−795.

(24) Psencík, J., Ikonen, T. P., Laurinmäki, P., Merckel, M. C., Butcher, S. J., Serimaa, R. E., and Tuma, R. (2004) Lamellar organization of pigments in chlorosomes, the light harvesting complexes of green photosynthetic bacteria. *Biophys. J. 87*, 1165−1172.

(25) Psencík, J., Arellano, J. B., Ikonen, T. P., Borrego, C. M., Laurinmäki, P. A., Butcher, S. J., Serimaa, R. E., and Tuma, R. (2006) Internal structure of chlorosomes from brown-colored *Chlorobium* species and the role of carotenoids in their assembly. *Biophys. J. 91*, 1433−1440.

(26) Arellano, J.B., Torkkeli, M., Tuma, R., Laurinmäki, P., Melo, T. B., Ikonen, T. P., Butcher, S. J., Serimaa, R. E., and Psencík, J. (2008) Hexanol-induced order-disorder transitions in lamellar self-assembling aggregates of bacteriochlorophyll *c* in *Chlorobium tepidum* chlorosomes. *Langmuir 24*, 2035−2041.

(27) Oostergetel, G. T., Reus, M., Chew, A. G. M., Bryant, D. A., Boekema, E. J., and Holzwarth, A. R. (2007) Long-range organization of bacteriochlorophyll in chlorosomes of *Chlorobium tepidum* investigated by cryo-electron microscopy. *FEBS Lett. 581*, 5435−5439.

(28) Ganapathy, S., Oostergetel, G. T., Wawrzyniak, P. K., Reus, M., Chew, A. G. M., Buda, F., Boekema, E. J., Bryant, D. A., Holzwarth, A. R., and de Groot, H. J. M. (2009) Alternating *syn-anti* bacteriochlorophylls form concentric helical nanotubes in chlorosomes. *Proc. Natl. Acad. Sci. U.S.A. 106*, 8525−8530.

(29) Larsen, K. L., Miller, M., and Cox, R. P. (1995) Incorporation of exogenous long-chain alcohols into bacteriochlorophyll *c* homologs by *Chloroflexus aurantiacus*. *Arch. Microbiol. 163*, 119−123.

(30) Steensgaard, D. B., Cox, R. P., and Miller, M. (1996) Manipulation of the bacteriochlorophyll *c* homolog distribution in the green sulfur bacterium *Chlorobium tepidum*. *Photosynth. Res. 48*, 385−393.

(31) Mizoguchi, T., and Tamiaki, H. (2007) The effect of esterifying chains at the 17-propionate of bacteriochlorophylls-*c* on their self-assembly. *Bull. Chem. Soc. Jpn. 80*, 2196−2202.

(32) Tamiaki, H., Miyata, S., Kureishi, Y., and Tanikaga, R. (1996) Aggregation of synthetic zinc chlorins with several esterified alkyl chains as models of bacteriochlorophyll-*c* homologs. *Tetrahedron 52*, 12421−12432.

(33) Huber, V., Katterle, M., Lysetska, M., and Würthner, F. (2005) Reversible self-organization of semisynthetic zinc chlorins into well-defined rod antennae. *Angew. Chem., Int. Ed. 44*, 3147−3151.

(34) Tamiaki, H., Michitsuji, T., and Shibata, R. (2008) Synthesis of zinc bacteriochlorophyll-*d* analogues with various 17-substituents and their self-aggregates in non-polar organic solvents. *Photochem. Photobiol. Sci. 7*, 1225−1230.

(35) Zupcanova, A., Arellano, J. B., Bina, D., Kopecky, J., Psencík, J., and Vacha, F. (2008) The length of esterifying alcohol affects the aggregation properties of chlorosomal bacteriochlorophylls. *Photochem. Photobiol. 84*, 1187−1194.

(36) Huber, V., Sengupta, S., and Würthner, F. (2008) Structure-property relationships for self-assembled zinc chlorin light-harvesting dye aggregates. *Chem.—Eur. J. 14*, 7791−7807.

(37) Uemura, S., Sengupta, S., and Würthner, F. (2009) Cyclic self-assembled structures of chlorophyll dyes on HOPG by the dendron wedge effect. *Angew. Chem., Int. Ed. 48*, 7825−7828.

(38) Ganapathy, S., Sengupta, S., Wawrzyniak, P. K., Huber, V., Buda, F., Baumeister, U., Würthner, F., and de Groot, H. J. M. (2009) Zinc chlorins for artificial light-harvesting self-assemble into antiparallel stacks forming a microcrystalline solid-state material. *Proc. Natl. Acad. Sci. U.S.A. 106*, 11472−11477.

(39) Saga, Y., Nakai, Y., and Tamiaki, H. (2009) Temperature-dependent spectral changes of self-aggregates of zinc chlorophylls esterified by different linear alcohols at the 17-propionate. *Supramol. Chem. 21*, 738−746.

(40) Psencík, J., Torkkeli, M., Zupcanova, A., Vacha, F., Serimaa, R. E., and Tuma, R. (2010) The lamellar spacing in self-assembling bacteriochlorophyll aggregates is proportional to the length of the esterifying alcohol. *Photosynth. Res. 104*, 211−219.

(41) Miyatake, T., Tamiaki, H., Shinoda, H., Fujiwara, M., and Matsushita, T. (2002) Synthesis and self-assembly of amphiphilic zinc chlorins possessing a 3^1-hydroxy group. *Tetrahedron 58*, 9989−10000.

(42) Miyatake, T., Tanigawa, S., Kato, S., and Tamiaki, H. (2007) Aqueous self-aggregates of amphiphilic zinc 3^1-hydroxy- and 3^1-methoxy-chlorins for supramolecular light-harvesting systems. *Tetrahedron Lett. 48*, 2251−2254.

(43) Saga, Y., Nakagawa, T., Miyatake, T., and Tamiaki, H. (2009) Changes of aqueous self-assemblies of zinc chlorophyll derivatives possessing a hydrophilic chain by treatment with organic solvents. *Chem. Lett. 38*, 882−883.

(44) Tamiaki, H., Shinkai, A., and Kataoka, Y. (2009) Synthesis of galactosylated zinc bacteriochlorophyll-*d* analogs and their self-aggregation in an aqueous methanol solution. *J. Photochem. Photobiol., A 207*, 115−125.

(45) Shibata, R., and Tamiaki, H. (2006) Self-aggregation of synthetic zinc chlorophyll derivative possessing a perfluoroalkyl group in a fluorinated solvent. *Bioorg. Med. Chem. 14*, 2235−2241.

(46) Tamiaki, H., Nishiyama, T., and Shibata, R. (2007) Self-aggregation of zinc chlorophylls possessing perfluoroalkyl chains in fluorous solvents: Selective extraction of the self-aggregates with fluorous phase and accelerated formation of the ordered supramolecules in this phase. *Bioorg. Med. Chem. Lett. 17*, 1920−1923.

(47) Shibata, R., Mizoguchi, T., Inazu, T., and Tamiaki, H. (2007) Self-aggregation of synthetic zinc chlorophyll derivatives possessing multi-perfluoroalkyl chains in perfluorinated solvents. *Photochem. Photobiol. Sci. 6*, 749−757.

(48) Shibata, R., Koike, K., Hori, H., and Tamiaki, H. (2008) Self-aggregation of synthetic zinc chlorophyll derivatives possessing fluoroalkyl chains in liquid carbon dioxide as models of green photosynthetic bacterial antennae. *Chem. Lett. 37*, 532−533.

(49) Röger, C., Müller, M. G., Lysetska, M., Miloslavina, Y., Holzwarth, A. R., and Würthner, F. (2006) Efficient energy transfer from peripheral chromophores to the self-assembled zinc chlorin rod antenna: A bioinspired light-harvesting system to bridge the "green gap". *J. Am. Chem. Soc. 128*, 6542−6543.

(50) Röger, C., Miloslavina, Y., Brunner, D., Holzwarth, A. R., and Würthner, F. (2008) Self-assembled zinc chlorin rod antennae powered by peripheral light-harvesting chromophores. *J. Am. Chem. Soc. 130*, 5929−5939.

(51) Saga, Y., Osumi, S., Higuchi, H., and Tamiaki, H. (2005) Bacteriochlorophyll-*c* homolog composition in green sulfur photosynthetic bacterium *Chlorobium vibrioforme* dependent on the concentration of sodium sulfide in liquid cultures. *Photosynth. Res. 86*, 123−130.

(52) Saga, Y., Harada, J., Hattori, H., Kaihara, K., Hirai, Y., Oh-oka, H., and Tamiaki, H. (2008) Spectroscopic properties and bacteriochlorophyll *c* isomer composition of extramembranous light-harvesting complexes in the green sulfur photosynthetic bacterium *Chlorobium tepidum* and its *CT0388*-deleted mutant under vitamin B_{12}-limited conditions. *Photochem. Photobiol. Sci. 7*, 1210−1215.

(53) Saga, Y., and Tamiaki, H. (2004) Comparison between chlorosomes containing bacteriochlorophyll-*c* and chlorosomes containing bacteriochlorophyll-*d* isolated from two substrains of green sulfur photosynthetic bacterium *Chlorobium vibrioforme* NCIB 8327. *J. Photochem. Photobiol., B 75*, 89−97.

(54) Hirai, Y., Tamiaki, H., Kashimura, S., and Saga, Y. (2009) Physicochemical studies of demetalation of light-harvesting bacteriochlorophyll isomers purified from green sulfur photosynthetic bacteria. *Photochem. Photobiol. 85*, 1140−1146.

(55) Kobayashi, M., Oh-oka, H., Akutsu, S., Akiyama, M., Tominaga, K., Kise, H., Nishida, F., Watanabe, T., Amesz, J., Koizumi, M., Ishida, N., and Kano, H. (2000) The primary electron acceptor of green sulfur bacteria, bacteriochlorophyll 663, is chlorophyll *a* esterified with Δ2,6-phytadienol. *Photosynth. Res. 63*, 269−280.

(56) Chew, A. G. M., Frigaard, N.-U., and Bryant, D. A. (2007) Bacteriochlorophyllide *c* C-8^2 and C-12^1 methyltransferases are essential for adaptation to low light in *Chlorobaculum tepidum*. *J. Bacteriol. 189*, 6176−6184.