

The Effect of Esterifying Chains at the 17-Propionate of Bacteriochlorophylls-*c* on Their Self-Assembly

Tadashi Mizoguchi and Hitoshi Tamiaki*

Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu 525-8577

Received April 24, 2007; E-mail: tamiaki@se.ritsumei.ac.jp

Bacteriochlorophyll(BChl)-*c* analogs having several esterifying chains at their 17-propionate were synthesized by two procedures. BChls-*c* with shorter chains (C_1 and C_4) on the 17-substituent were synthesized by transesterification under mild basic conditions, while those with longer chains (C_8 and C_{16}) were obtained by the method of supplementation of exogenous alcohols in the cultures of *Chlorobium tepidum*. All the BChl-*c* analogs self-organized to form their micelle-like aggregates in an aqueous solution containing Triton X-100 as a surfactant. To investigate the effect of the length of the esterifying chains, these micelle-like aggregates were characterized by electronic absorption, resonance Raman and dynamic light scattering measurements, and by chemical dissociation of the aggregates. The results indicated that the length of the esterifying chains affected stabilization of the self-assembly of BChls-*c* but neither construction of the aggregates nor their overall sizes.

All naturally occurring (bacterio)chlorophyll ((B)Chl) pigments are synthesized in photosynthetic organisms.[1,2] Such (B)Chl molecules have a propionate-type ester group at their 17-position, except most Chls-*c*.[3–5] The ester groups are not directly conjugated with the π-systems in (B)Chl molecules and do not affect the electronic absorption spectra of their monomeric forms. As a result, they have attracted little attention in comparison with other peripheral substituents. However, it is assumed that the esters play a key role in stabilizing and/or regulating supramolecular structures (=aggregates of (B)Chls) in photosynthetic apparatuses.

There are two methods to make (B)Chls aggregated in natural systems. One is based on the pigment-to-protein interaction found in any phototrophs. Typically, X-ray crystallography of antennae of purple photosynthetic bacteria revealed the atomic structure of cyclic aggregates of BChl-*a* on transmembranous peptides.[6] In the antenna, the BChl molecules were bound to the proteins through coordination bonds between histidine residues in oligopeptides and divalent magnesium atoms in BChls. Their absorption spectra show longer wavelength shifts than those in organic solvents. In the system, the long esterifying chains at the 17-propionate are believed to stabilize complexation of oligopeptides with BChl-*a* molecules.

The other method for aggregation of (B)Chls is based on pigment-to-pigment interaction found in the unique antennae of green photosynthetic bacteria, so-called chlorosomes.[7–11] The supramolecular structure at an atomic level has not been clarified, although electron micrography indicated the presence of rod-like architecture in chlorosomes.[12–14] The rod consists of well-ordered aggregates of chlorosomal BChls (BChls-*c*, -*d*, and -*e*, see Scheme 1) without proteins, in sharp contrast to the above purple bacterial antennae. Recently, Pšenčik and co-workers claimed that the supramolecular structures in chlorosomes were lamellar organizations.[15,16] Their absorption spectra show longer wavelength shifts than those in organic solvents. BChls-*c*, -*d*, and -*e* found in chlorosomes have functional groups which enhance hydrogen bonding and coordination bonding along their Qy axes. These bondings along the Qy axis have been well-studied and are known to be crucial for the self-aggregation of BChls-*c*, -*d*, and -*e*.[7–11,17] On the other hand, the function of the long esterifying chains on the 17-position along the Qx axis has been believed to stabilize the rod-like supramolecular structures in chlorosomes.

To elucidate the precise role of the 17^2-esters in chlorosomal BChls, numerous attempts have been made using both natural[18] and synthetic type compounds [Ref. 5 for the comprehensive review and the references cited therein]. As examples, examination of the alteration of the 17^2-esters inside chlorosomes was good to understand the effect of the esters on chlorosomal self-assembly.[19,20] In *Chlorobium (Chl.) tepidum*, a farnesyl (C_{15}) group was exclusively observed for the ester of BChl-*c*. When exogenous alcohols were added to the cultures, the corresponding esters of BChl-*c* were produced instead of the ordinary alcohol (farnesol).[20] Longer branched phytyl (C_{20}) and geranylgeranyl (C_{20}) as well as other non-branched octadecanyl (C_{18}) and dodecyl (C_{12}) alcohols were partially incorporated to BChl-*c*. Only the cultures supplemented by dodecyl alcohol showed the 6 nm blue shifted Qy absorption compared with those unsupplemented. Thus, shortening the ester chain ($C_{15} \rightarrow C_{12}$) would affect the optical properties of supramolecular self-aggregates in chlorosomes. However, other changes (additional shortening of the 17^2-esters) could not be applied in the above modification due to the toxicity of exogenous alcohols supplemented.

Synthetic zinc chlorins possessing the primary alcoholic OH group at the 3^1-position have also been used to investigate the function of the 17^2-esters to chlorosomal self-assembly.[8,21] The interactive hydroxy group of primary alcohols is less sterically hindered than the secondary alcoholic OH in usual chlorosomal BChls possessing 1-hydroxyethyl group at the

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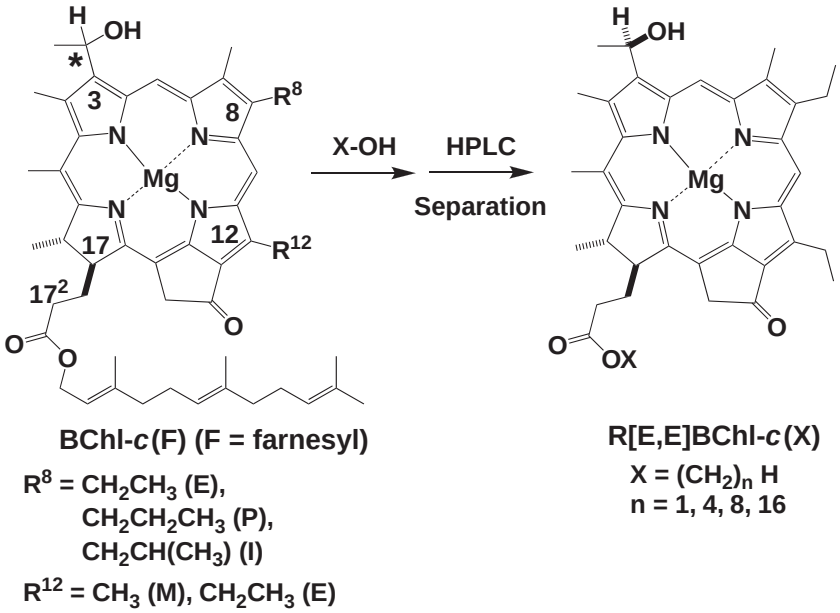

Scheme 1. The ester exchange reaction for BChls-*c*(X) (X = C_1, C_4, C_8, and C_{16}). "X" in parentheses indicates the ester group at the 17-propionate.

3-position. Therefore, these synthetic compounds self-aggregated more tightly, showing that the change of esters was not sensitive to the self-assembly.[5,22] Recently, the effect of numbers of the long esterifying groups on artificial self-aggregation has been demonstrated. For example, Würthner and his colleagues reported that synthetic zinc chlorin possessing two dodecyl groups self-aggregated in hexane containing THF.[23] The self-aggregates gave rod-like supramolecular architectures on the basis of atomic force microscopic analysis. They claimed that the multiple chains in a molecule would be important for production of the rod-like supramolecules. This has also been confirmed using fluorinated zinc chlorins possessing multiple-perfluorinated long chains on the 17-position.[24,25]

In this study, we synthesized natural type BChls-*c* possessing several esterifying chains at the 17-propionate as well as a 1-hydroxyethyl group at the 3-position and divalent central magnesium by two procedures. BChls-*c* having shorter chains at the 17-propionate (carbon numbers of 1 and 4) were efficiently synthesized by transesterification under mild basic conditions. On the other hand, BChls-*c* having longer chains (carbon numbers of 8 and 16) at the position were obtained by supplementation of exogenous alcohols in the cultures of *Chl. tepidum*. A series of BChls-*c* having several esterifying chains thus obtained self-organized to form the micelle-like aggregates in an aqueous solution containing Triton X-100 as a surfactant. The effect of the length of the esterifying chains in BChls-*c* (C_1, C_4, C_8, C_{16}, and natural farnesyl (C_{15})) upon their self-assembly was systematically investigated by means of electronic absorption, resonance Raman and dynamic light scattering measurements, and by chemical dissociation of the self-aggregates.

Results and Discussion

Molecular Structures of BChls-*c* and Their Self-Assembly in Chlorosomes. Molecular Structures of BChls-*c*: The left-hand drawing in Scheme 1 depicts the molecular structure of naturally occurring BChl-*c* from *Chl. tepidum*. The intact BChl-*c* has a C_{15} farnesyl (F) group as the 17-propionate ester, and is a mixture of both epimers at the chiral 3^1-position (indicated by an asterisk) and several homologs differing by the degree of methylation at the 8^2- and 12^1-positions (R^8 and R^{12}). In the present investigation, we use enantiomerically and homologously pure R[E,E]BChl-*c*(X) isolated by HPLC as shown in the right-hand drawing of Scheme 1, since the compound is always the main component of the above mixture (≈60% of total BChls-*c*). Here, "X" in parentheses means the propionate ester group at the 17-position. Moreover, naturally occurring farnesylated (3^1R)-epimers having 8-ethyl (E) and 12-methyl (M) groups, 8/12-E groups, 8-propyl (P)/12-E groups and 8-isobutyl (I)/12-E groups are termed R[E,M]BChl-*c*(F), R[E,E]BChl-*c*(F), R[P,E]BChl-*c*(F), and R[I,E]BChl-*c*(F), respectively, and (3^1S)-epimers are also named similarly except for change of R to S at the chiral 3^1-position.

Electronic Absorption Spectra of Chlorosomes: Figure 1 shows the electronic absorption spectrum of chlorosomes from *Chl. tepidum* in 50 mM Tris-HCl buffer (pH 8.0) (solid) and that of monomeric isolated BChls-*c*(F) in 50 mM Tris-HCl buffer (pH 8.0) containing 0.100% (wt/vol) Triton X-100 (dotted). Chlorosomes are characterized by their far-red shifted Qy-absorption around 740 nm due to the self-aggregation of their composite BChls-*c*(F) under a hydrophobic environment made by a chlorosomal lipid monolayer. The shoulder appearing around 500 nm in chlorosomes is ascribable to the absorption of carotenoids.

Synthesis of BChls-*c*(X). In Vitro Transesterification: An epimerical and homologous mixture of BChls-*c*(F) from *Chl. tepidum* was changed to other esters by treatment of an appropriate alcohol X-OH and K_2CO_3 as shown in Scheme 1.[26] The reaction was applied for shorter alcohols like methanol (C_1) and 1-butanol (C_4) but not for longer ones including 1-octanol (C_8) and 1-hexadecanol (C_{16}) because the

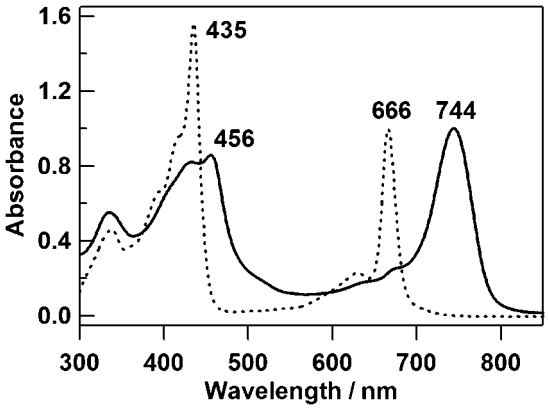

Fig. 1. Electronic absorption spectra of chlorosomes from *Chl. tepidum* in 50 mM Tris-HCl buffer (pH 8.0) (solid) and isolated BChls-*c*(F) in 50 mM Tris-HCl buffer (pH 8.0) containing 0.100% (wt/vol) Triton X-100 (dotted).

latter were less reactive (vide infra). BChls-*c*(X) (X = C_1 and C_4) were obtained in yields of ≈100% and ≈88%, respectively. It was possible to change the farnesyl to hexyl ester with 1-hexanol (C_6) in ≈60% yield according to this method. However, the method is not suitable for the preparation of BChls-*c*(X) having a longer hydrocarbon chain (X > C_6) due to a decrease of the reactivity and the troublesome removal of unreacted alcohols with high boiling points. Therefore, an alternative method is necessary for facile preparation of BChls-*c*(X) having a longer hydrocarbon chain (X = C_8 and C_{16}) at the 17-propionate. The sterically less hindered 1-hydroxyethyl group at the 3-position of BChl-*c* did not react intermolecularly with the propionate under the mild basic conditions examined here. This was confirmed by LC-MS analysis as described below.

In Vivo Transesterification: Esterification of the 17-propionate acid moiety of (bacterio)chlorophyllides catalyzed by enzyme (B)Chl synthase occurs in the final stage of (B)Chl biosynthesis. When an appropriate alcohol is supplemented in a green photosynthetic bacterial culture, incorporation of an added alcohol into chlorosomal BChl molecules has been expected and already demonstrated for BChl-*c* from *Chl. tepidum* and *Chloroflexus aurantiacus*.[19,20] This method is not used for shorter alcohols (X = C_1–C_6), since exogenous alcohols are potentially toxic to their bacterial growth. Therefore, this method was applied for C_8 and C_{16} alcohols, and the corresponding BChls-*c*(X) could be obtained in the relative yields of ≈39% for C_8 and ≈37% for C_{16} in the total BChls-*c* produced. Figure 2a shows the HPLC profiles of the pigments extracted from the cultured cells with (solid) and without supplementation of exogenous C_8-alcohol (dotted). We can clearly see the additional peaks at the retention times of 3.9, 4.2, 4.7, and 5.3 min by the supplementation. These are assigned to the desirable homologous mixture of BChls-*c*(X) (X = C_8) on the basis of LC-MS analysis as described below. The supplementation of the branched alcohols, geranyl (C_8 in the linear length plus two methyl groups) and geranylgeranyl (C_{16} in the length plus four methyl groups) was also performed, but the incorporation was less adequate than that of the corresponding non-branched alcohols (C_8 and C_{16}).

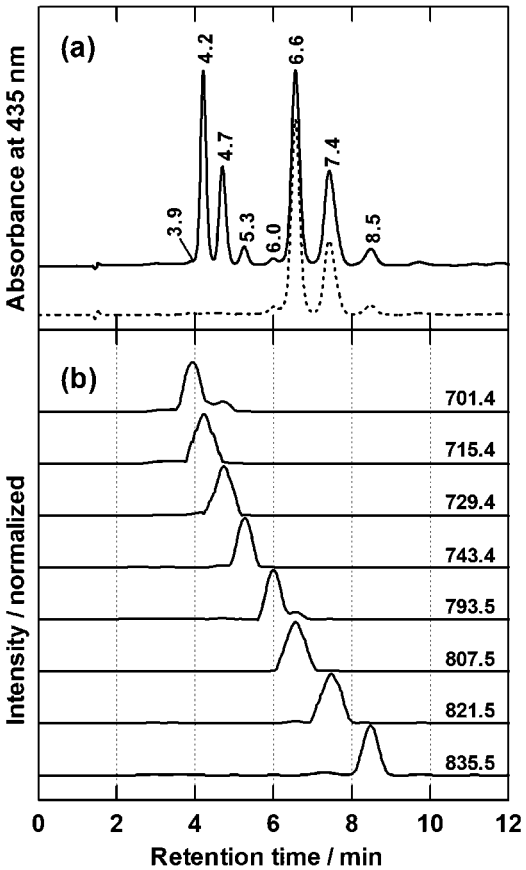

Fig. 2. HPLC profiles of the pigment extracts from the cells with (solid) and without supplementation of exogenous C_8-alcohol (dotted) (a), together with the corresponding mass-chromatograms sliced at each molecular weight of BChls-*c*(X) (X = C_8 and F) (b). The $[MH]^+$ of R[E,E]-BChls-*c*(X) is calculated for 715.41 (X = C_8) and 807.47 (X = F).

Characterization of Synthetic BChls-*c*(X) by APCI-LC-MS Analysis. All the synthetic BChls-*c*(X) were confirmed using on-line atmospheric pressure chemical ionization (APCI) LC-MS analysis, since BChls-*c*(F) from *Chl. tepidum* as starting materials had been well-defined. In the present in vivo and in vitro synthesis, only the 17-propionate ester groups were modified from BChls-*c*(F) fully determined by mass spectrometry and NMR spectroscopy.[26] Therefore, LC-MS is the most powerful tool to identify such structural differences. Figure 2 shows the typical result of LC-MS analysis of BChls-*c*(X) (X = C_8) obtained by the method of supplementation of exogenous alcohol. This clearly demonstrated that the ester exchange afforded the desired products without degradation. Four ester exchanged BChls-*c*(X) (X = C_8) were detected at the retention times of 3.9, 4.2, 4.7, and 5.3 min and were clearly assigned as R[E,M] ($[MH]^+$ = 701.4), R[E,E] ($[MH]^+$ = 715.4), R/S[P,E] ($[MH]^+$ = 729.4), and S[I,E] ($[MH]^+$ = 743.4) in the order of elution on the basis of the corresponding mass-chromatograms shown in Fig. 2b. Four unreacted BChls-*c* having a farnesyl group were also confirmed at the retention times of 6.0, 6.6, 7.4, and 8.5 min as R[E,M] ($[MH]^+$ = 793.5), R[E,E] ($[MH]^+$ = 807.5), R/S[P,E] ($[MH]^+$ = 821.5), and S[I,E] ($[MH]^+$ = 835.5) in the order of elution.

Table 1. APCI-Mass Spectrometric Results of R[E,E]BChls-*c*(X) Possessing Various 17-Propionate Esters

R[E,E]BChls-*c*(X)	X-OH	Chemical formula	Calcd. for $[MH]^+$	Observed ions		
				$[MH]^+$	$[MH + CH_3OH]^+$	$[MH - H_2O]^+$
X = C_1	methanol	$C_{36}H_{40}MgN_4O_4$	617.30	617.1	649.1	599.1
X = C_4	1-butanol	$C_{39}H_{46}MgN_4O_4$	659.35	659.1	691.1	641.1
X = C_8	1-octanol	$C_{43}H_{54}MgN_4O_4$	715.41	715.3	747.3	697.2
X = farnesyl (C_{15})	farnesol	$C_{50}H_{62}MgN_4O_4$	807.47	807.3	839.3	789.3
X = C_{16}	1-hexadecanol	$C_{51}H_{70}MgN_4O_4$	827.53	827.3	859.3	809.4

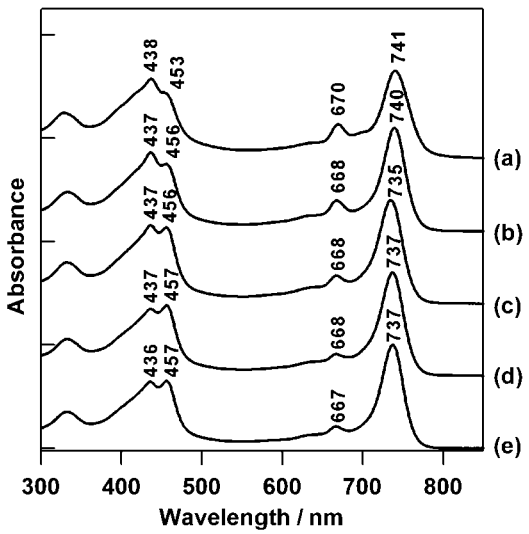

Fig. 3. Electronic absorption spectra of the micelle-like aggregates of R[E,E]BChls-*c*(X) (ca. 2.0×10^{-5} M) in 50 mM Tris-HCl buffer (pH 8.0) containing 0.020% (wt/vol) Triton X-100: (a) X = C_1, (b) X = C_4, (c) X = C_8, (d) X = F, and (e) X = C_{16}.

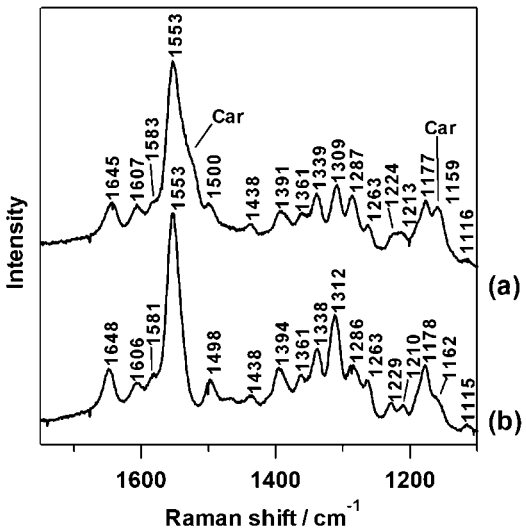

Fig. 4. Resonance Raman spectra of chlorosomes from *Chl. tepidum* in 50 mM Tris-HCl buffer (pH 8.0) (a) and the micelle-like aggregates of R[E,E]BChl-*c*(X) (X = C_{16}) in 50 mM Tris-HCl buffer (pH 8.0) containing 0.020% (wt/vol) Triton X-100 (b) excited at 457.9 nm. Carotenoids in chlorosomes are indicated by Car.

We also obtained the mass spectra of each peak simultaneously. Table 1 summarizes the results of mass spectrometric analysis thus obtained. All the BChls-*c*(X) gave their molecular-ion peaks (a protonated form, $[MH]^+$) as their main peaks. Two additional peaks were also observed: one was $[MH + 32]^+$, and the other was $[MH - 18]^+$. These are identified as a MeOH adduct to protonated BChl-*c* species and an H_2O loss from the MH^+ species. The latter fragment ion, $[MH - H_2O]^+$, is useful to confirm the reservation of a 1-hydroxyethyl group at the 3-position of BChls-*c* during the ester exchange. The assignment is consistent with the literature.[27]

Electronic Absorption Spectra of Micelle-Like Aggregates. As mentioned in the above section, we obtained a series of R[E,E]BChls-*c*(X) (X = C_1, C_4, C_8, natural F (C_{15}), and C_{16}). To investigate the effect of the esterifying chains at the 17-propionate of BChls-*c* on their self-assembly, we used a Triton X-100 micelle-system,[28] because the self-assembly in natural chlorosomes occurred in a hydrophobic environment made by a lipid monolayer.

Figure 3 shows the electronic absorption spectra of HPLC-isolated R[E,E]BChls-*c*(X) in an aqueous 50 mM Tris-HCl buffer (pH 8.0) containing 0.020% (wt/vol) Triton X-100 at the concentration of ca. 2.0×10^{-5} M: (a) X = C_1, (b) X = C_4, (c) X = C_8, (d) X = F, and (e) X = C_{16}. Similarly to the absorption spectrum of chlorosomes shown in Fig. 1, all the BChls-*c*(X) had the characteristic far-red shifted Qy-absorptions around 740 nm and self-aggregated in the aqueous solution. The shifts of Qy-absorption maxima found in these micelle-like aggregates were slightly smaller than that of chlorosomes. This might be ascribable to the different pigment compositions between chlorosomes (a mixture of epimers and homologs) and the micelle-like aggregates (pure R[E,E]).[29–31] When these micelle-like aggregates were incubated for 1-day at room temperature, their sedimentation was observed for X = C_1 and C_4. On the other hand, no sediment had been observed for X = C_8, F or C_{16} with prolonged incubation. These observations clearly indicate that the length of the 17-propionate esters affects stabilization of chlorosome-like supramolecules. The micelle-like aggregates of R[E,E]BChl-*c*(X) (X = C_6) were also sedimented (data not shown). Therefore, at least a length of C_8 for the esterifying chains is required to stabilize the supramolecular structures through the hydrophobic interaction among the long chains of the esters and Triton X-100.

Resonance Raman Spectra of the Self-Aggregates of BChls-*c*(X). Figure 4 shows resonance Raman spectra (excited at 457.9 nm) of chlorosomes (a) and the micelle-like aggregates composed of R[E,E]BChl-*c*(X) (X = C_{16}) (b). These

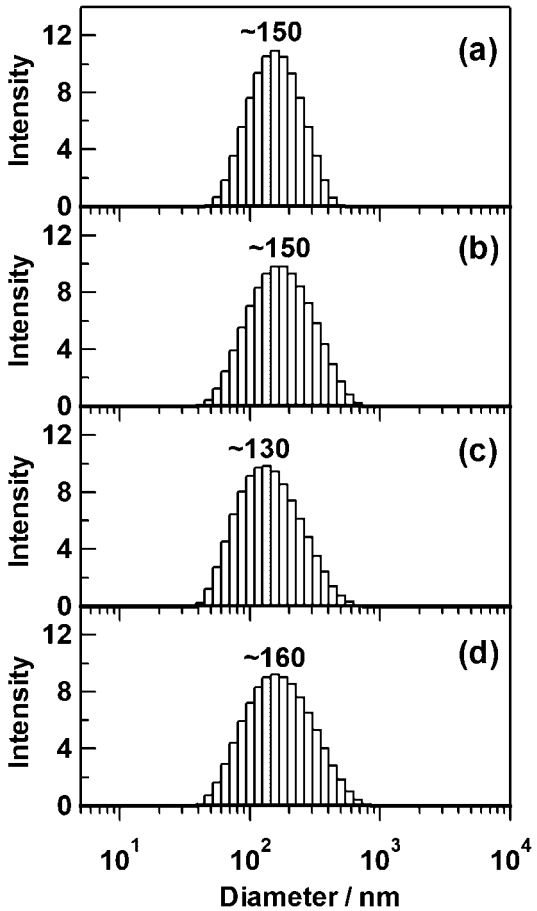

Fig. 5. Dynamic light scattering profiles of chlorosomes from *Chl. tepidum* (a) and the micelle-like aggregates of R[E,E]BChls-*c*(X) (b) X = C_8, (c) X = F, and (d) X = C_{16}.

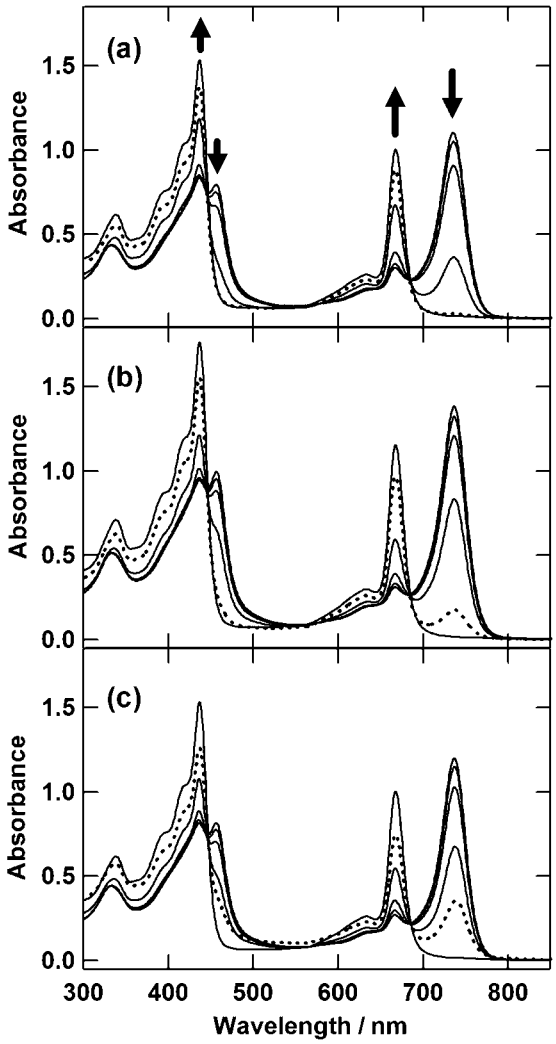

Fig. 6. Changes in electronic absorption spectra of the micelle-like aggregates of R[E,E]BChls-*c*(X) upon titration of 1-hexanol (0 → 2.0 → 4.0 → 6.0 → 8.0 → 10 μL to 2.0 mL aggregated solution): (a) X = C_8, (b) X = F, and (c) X = C_{16}. Arrows indicate the absorption changes with increasing amount of titrated 1-hexanol. Dotted lines correspond to the absorption spectra titrated with 8.0 μL 1-hexanol.

Raman spectra were found to be quite similar over the investigated region (1100–1750 cm^{-1}). The slight differences between the spectra in their intensity are ascribable to the different pigment compositions between chlorosomes (a mixture of epimerical and homologous BChls-*c* and carotenoids) and the micelle-like aggregates (pure R[E,E]BChl-*c*). The bands around 1520 and 1160 cm^{-1} in chlorosomes were assigned to carotenoids.[32,33] According to the earlier Raman studies on chlorosomes, the self-aggregates of BChls-*c* in chlorosomes gave the characteristic vibrational bands due to their self-aggregation: the hydrogen bonded 13-C=O and the pentacoordinated skeletal bands were assigned to 1645 and 1553 cm^{-1}, respectively.[32–34] In the micelle-like aggregates, the above bands were clearly observed at 1648 and 1553 cm^{-1}. The Raman spectra of other BChls-*c*(X) having different 17^2-esters were also recorded and were found to be identical to that of R[E,E]BChl-*c*(X) (X = C_{16}) (data not shown). These results indicated that the length of the esterifying chains at the 17-propionate was not sensitive to form chlorosomal local interaction.

Size Distributions Determined by Dynamic Light Scattering. Figure 5 shows the profiles of size distribution that were determined by dynamic light scattering. The hydrodynamic diameter at the maximum population is ≈150 nm in chlorosomes (a), while it is ≈150, ≈130, and ≈160 nm in the micelle-like aggregates composed of R[E,E]BChl-*c*(X) having X = C_8 (b), X = F (c), and X = C_{16} (d). These diameters and size distribution are quite similar to each other. Therefore, the length of the 17-propionate was also insensitive to their overall sizes.

Chemical Dissociation of Self-Aggregates by Titration of 1-Hexanol. To analyze stabilities of the self-aggregates of BChls-*c*(X) having different 17-propionates under an external perturbation, titration of a hydrophobic alcohol, 1-hexanol (solubility in water at ca. 59 mM), to the micelle-like suspensions was applied. Figure 6 shows changes in the absorption spectra of R[E,E]BChls-*c*(X) having X = C_8 (a), X = F (b), and X = C_{16} (c) with the addition of 1-hexanol. Each spectrum in the figure corresponds to the stepwise addition of 1-hexanol: 0 → 10 μL at a 2.0 μL interval to 2.0 mL of the

aggregated solutions. Arrows indicate the absorption change with the addition of 1-hexanol. Clear isosbestic points indicate the presence of two species in the visible spectral change: equilibrium between the aggregates and the monomer of BChl-*c*. When 8.0 μL 1-hexanol was added to the aggregates of BChl-*c*(X) (X = C_8), the red-shifted Qy absorption band almost disappeared as shown by the dotted line in Fig. 6a. However, the red-shifted Qy absorption of BChls-*c*(X) having X = F or X = C_{16} retained about 13% or 30% of the original absorbance, respectively, with the addition of 8.0 μL 1-hexanol (see the dotted lines in Figs. 6b and 6c). These results strongly indicate that the self-aggregates composed of BChls-*c*(X) having longer chains as their 17-propionate are more stabilized than those having shorter chains (X ≤ C_8) through the hydrophobic interaction among the long chains of the esters and Triton X-100.

In this study, we synthesized natural type BChl-*c*(X) analogs having several esterifying chains at the 17-propionate as well as a chiral 1-hydroxyethyl group at the 3-position and central magnesium by two procedures. BChls-*c*(X) having shorter chains at the position (X = C_1 and C_4) were synthesized by transesterification under mild basic conditions, while those having longer chains (X = C_8 and C_{16}) were obtained by the method of supplementation of exogenous alcohols in the bacterial cultures. The effect of the length of the esterifying chains upon the self-aggregation of BChls-*c*(X) was systematically examined in an aqueous solution containing 0.020% (wt/vol) Triton X-100 as a surfactant by means of electronic absorption, resonance Raman and dynamic light scattering measurements, and by chemical dissociation of the self-aggregates. All the BChls-*c*(X) (X = C_1, C_4, C_8, natural F (C_{15}), and C_{16}) self-organized into the micelle-like aggregates in the aqueous solution. These micelle-like aggregates are almost identical to the intact chlorosomes in terms of their optical properties and overall sizes. However, at least esterifying chains with a length of X = C_8 is required to stabilize the self-assembly of BChls-*c*(X) through the hydrophobic interaction among the long chains of the 17-propionates and Triton X-100. Moreover, the stabilization effect of the self-assembly was pronounced by increasing the length of the 17^2-esters (C_8 → C_{15} (F) → C_{16}).

Experimental

General. Electronic absorption spectra were measured with a Hitachi U-3500 spectrophotometer (Hitachi Ltd., Tokyo, Japan). Resonance Raman spectra were measured with a JASCO NR-1800 laser Raman spectrometer; solutions in a quartz cell were irradiated with 457.9 nm line of an Ar^+ laser (≈35 mW) (Spectra-Physics Stabilite-2017, Mountain View, USA). Raman data accumulated for 30 s were collected in the back-scattering geometry. Dynamic light scattering profiles were measured with a Malvern Zetasizer Nano-ZS (Malvern Instruments, Ltd., Worcestershire, UK). Optical density was about 1.0/10 mm at the Qy-absorption band for all the spectroscopic measurements (ca. 2.0×10^{-5} M). The LC-MS of the synthetic BChls-*c* was performed using a Shimadzu LCMS-2010EV system (Shimadzu, Kyoto, Japan) equipped with an APCI probe as described previously.[35] Analytical and preparative HPLC were performed using reverse-phase chromatography under the following conditions: For the analysis, column, Cosmosil 5C18-AR-II (3.0 × 150 mm, Nacalai Tesque, Kyoto, Japan); eluent, 5% (vol/vol) H_2O in methanol; flow rate, 0.5 mL min^{-1}; and detection wavelength, 435 nm, and for the preparation, column, Cosmosil 5C18-AR-II (10.0 × 250 mm); eluent, 10% (vol/vol) H_2O in methanol; flow rate, 3.0 mL min^{-1}; and detection wavelength, 455 nm. All the solvents were used without further purification.

Culturing of Green Sulfur Bacteria and Isolation of Their Chlorosomes. *Chl. tepidum* strain ATCC49652, a thermophilic green sulfur bacterium, was cultured at 43 °C anaerobically as described previously.[36] Chlorosomes are prepared from the harvested cells in 2 M NaSCN, 10 mM potassium phosphate (pH 7.4), and 10 mM sodium ascorbate by the method of Gerola and Olson.[37]

Synthesis of BChls-*c*(X). In Vitro Synthesis by Transesterification: Naturally occurring BChls-*c*(F) were prepared as described previously.[30] Ester exchange of the farnesyl group to desirable groups was performed on the basis of the reported procedure.[26] Briefly, BChls-*c*(F) (≈20 mg) were dissolved in a desirable alcohol (methanol (C_1) or 1-butanol (C_4), 10 mL). K_2CO_3 in a catalytic amount was added to the reaction mixture at room temperature. After stirring overnight under N_2, the mixture was poured into CH_2Cl_2, washed with water repeatedly, dried over Na_2SO_4, and concentrated to almost dryness. The residue was chromatographed (crystalline cellulose as an adsorbent) with hexane and acetone to remove the unreacted alcohol. Then, R[E,E]BChl-*c*(X) (X = C_1 and C_4) was isolated by HPLC and simultaneously characterized by APCI-LC-MS analysis. Their purities were also confirmed to be almost 100% by HPLC.

In Vivo Synthesis by Supplementation of Exogenous Alcohols: Incorporation of exogenous alcohols into BChl-*c* was performed by the method of Miller and co-workers.[19,20] The incorporation was applied for 1-octanol (C_8) and 1-hexadecanol (C_{16}), but BChls-*c*(X) having X = C_1 and C_4 esters could not be obtained by this method due to the inhibition of bacterial growth. Briefly, the stock alcohol emulsions containing 0.1% (vol/vol) appropriate alcohols were prepared, and then a small amount of the emulsion (4.0 mL) was successfully supplemented in the culture (50 mL) six times at 1-day intervals. The growth of photosynthetic bacteria was not inhibited at all. The pigments were extracted from the cultured cells which were treated with exogenous alcohols, as described previously.[30] The extracts were subjected to HPLC in order to determine the incorporation of the alcohols into BChl-*c* molecules. Simultaneously, each component was isolated by HPLC and confirmed by APCI-LC-MS analysis. The purity was also confirmed to be almost 100% by HPLC.

Preparation of Micelle-Like Aggregates. HPLC-isolated R[E,E]BChl-*c*(X) (X = C_1, C_4, C_8, natural F (C_{15}), and C_{16}) were dissolved in methanol containing Triton X-100 (Nacalai Tesque) at the concentration of 2.0×10^{-3} M, 50 μL of this solution was injected to 5.0 mL of Tris-HCl buffer (pH 8.0) (2.0×10^{-5} M) as described previously.[28] The concentration of Triton X-100 in the final solution was adjusted to be 0.020% (wt/vol) for preparation of the aggregates and 0.100% (wt/vol) for the monomer. The critical micellar concentration of Triton X-100 is estimated to be 0.015% (wt/vol). The suspension was homogenized for 10 s using a vortex mixer, and kept in the dark for more than 60 min at room temperature.

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