The 17-propionate esterifying variants of bacteriochlorophyll-*a* and bacteriopheophytin-*a* in purple photosynthetic bacteria

Tadashi Mizoguchi^a, Megumi Isaji^a, Jiro Harada^b, Yusuke Tsukatani^{c,d}, Hitoshi Tamiaki^{a,*}

^a *Graduate School of Life Sciences, Ritsumeikan University, Kusatsu, Shiga 525-8577, Japan*

^b *Department of Medical Biochemistry, Kurume University School of Medicine, Kurume, Fukuoka 830-0011, Japan*

^c *Earth-Life Science Institute, Tokyo Institute of Technology, Meguro, Tokyo 152-8550, Japan*

^d *PRESTO, Japan Science and Technology, Kawaguchi, Saitama 332-0012, Japan*

Keywords: bacteriochlorophyll-*a*; bacteriopheophytin-*a*; propionate-type ester group; purple bacteria; geranylgeranyl reductase; high-performance liquid chromatography.

Abbreviations: Alc., *Allochromatium*; APCI, atmospheric pressure chemical ionization; BChl, bacteriochlorophyll; BChlide, bacteriochlorophyllide; BPhe, bacteriopheophytin; BPheide, bacteriopheophorbide; Chl, chlorophyll; DHGG, dihydrogeranylgeranyl; *Erb.*, *Erythrobacter*; GG, geranylgeranyl; *Hlr.*, *Halorhodospira*; HPLC, high-performance liquid chromatography; LH, light-harvesting; NMR, nuclear

25 magnetic resonance; PDA, photodiode array; Phe, pheophytin; *Phs.*, *Phaeospirillum*;
26 *Phy*, phytyl; PS, photosystem; *Rba.*, *Rhodobacter*; RC, reaction-center; *Rcy.*,
27 *Rhodocyclus*; *Rdv.*, *Rhodovulum*; *Rfx.*, *Rhodoferrax*; *Rmi.*, *Rhodomicrobium*; *Rps.*,
28 *Rhodopseudomonas*; *Rsb.*, *Roseobacter*; *Rsp.*, *Rhodospirillum*; *Rvi.*, *Rubrivivax*; THGG,
29 tetrahydrogeranylgeranyl.

30 *Correspondence: Graduate School of Life Sciences, Ritsumeikan University,
31 Kusatsu, Shiga 525-8577, Japan. E-mail: tamiaki@fc.ritsumei.ac.jp, Fax:
32 +81-77-561-2659 (H. Tamiaki).

33

Abstract

Most purple photosynthetic bacteria contain bacteriochlorophyll(BChl)-*a* (a magnesium complex) and bacteriopheophytin(BPhe)-*a* (its free base) as their photoactive pigments. These pigments are composed of two parts: a cyclic tetrapyrrole as the chromophore and a long hydrocarbon-chain as the propionate-type esterifying group at the 17-position. The hydrocarbon-chain is usually an isoprenoid-type C₂₀ phytyl (Phy) group in both the pigments. In the ester group of BChl-*a*, several variants such as geranylgeranyl (GG), dihydrogeranylgeranyl (DHGG) and tetrahydrogeranylgeranyl (THGG) groups were found in the final stage of BChl-*a* biosynthesis. On the other hand, the esterifying variants in BPhe-*a* have not been studied as much due to the lower levels of this pigment relative to BChl-*a*. The esterifying group does not affect the electronic absorption properties of such pigments in the monomeric state, but drastically alters the hydrophobicity. In this study, BChl-*a* and BPhe-*a* in the six phylogenetically distinct classes of purple bacteria were analyzed in terms of their esterifying groups in the 17-propionate residues, using high-performance liquid chromatography. Both BChls-*a* and BPhe-*a* carrying GG, DHGG and THGG in addition to the usual Phy were found for all the bacterial species studied at measurable levels. In some of the species, the ratio of BPhe-*a* esterified with GG, DHGG and THGG over the total BPhe-*a* drastically decreased in comparison with that of the corresponding BChls-*a*. Especially, the relative content of BPhe-*a* with GG largely decreased. This observation might indicate that BPhe-*a* as a cofactor of reaction centers was preferentially esterified with partially reduced and flexible chains (THGG and Phy) rather than less reduced and rigid ones (GG and DHGG).

1. Introduction

Chlorophyll (Chl) and bacteriochlorophyll (BChl) pigments are essential components of the photosynthetic apparatus, and thus significant molecules for living organisms and environments. The pigments have their distinctive electronic absorption bands depending on molecular structures [1]. In purple photosynthetic bacteria, either BChl-*a* or BChl-*b* is selected to efficiently drive photosynthesis together with the corresponding demetalized bacteriopheophytin(BPhe)-*a* or *b* whose content is much lower than that of BChl [see Fig. 1(a)]. Other photosynthetic organisms, however, have structurally and optically different forms of (B)Chls to perform photosynthesis: Chl-*a*/pheophytin(Phe)-*a*/Chl-*b* in plants, Chl-*a*/Phe-*a*/Chl-*c* in certain algae, BChl-*g*/8¹-OH-Chl-*a* in heliobacteria and BChl-*a*/Chl-*a*/BChl-*c* in green sulfur bacteria as examples [1-3]. The BChl pigments found in purple bacteria are characterized as a partially reduced porphyrin skeleton termed bacteriochlorin (7,8-*trans*-17,18-*trans*-tetrahydroporphyrin) and the presence of a π -conjugated acetyl group at the 3-position as shown in Fig. 1(a); this is in comparison with the most recognized and abundant Chl-*a* (with a less reduced 17,18-*trans*-dihydroporphyrin and a vinyl group at the 3-position) in most oxygenic photosynthetic organisms. These structural features lead to a shift in their Q_y-absorption maxima up to a near infrared region: 771 nm (750 nm) for BChl-*a* (BPhe-*a*) and 795 nm (778 nm) for BChl-*b* (BPhe-*b*). BChl-*a* or BChl-*b* functions both in light-harvesting (LH) and reaction-center (RC) complexes, while its demetalized BPhe-*a* or BPhe-*b* localizes only in RCs and serves as the primary electron accepting component.

[Insert Figure 1]

Almost all naturally occurring (B)Chl pigments are esterified with various long

hydrocarbon-chains as their propionate-type esterifying groups at the 17-position: 17-CH₂-CH₂-COOR [see Fig. 1(a)]. Here, “R” is usually the isoprenoid type C₂₀ phytyl (Phy). The ester groups are not directly conjugated with the characteristic π -systems of (B)Chl pigments and do not affect the electronic absorption properties of their monomeric states. However, it was reported that removal of the phytyl group in (B)Chl molecules to form the corresponding free carboxylic acid induced a limited effect on the absorption spectra [4]. Thus, structures, functions and variations of the esters in (B)Chls have been less studied. However, the ester groups account for about 30% of the total molecular weight of (B)Chls, and thus are believed to play a key role in constructing functional pigment-protein complexes (LHs and/or RCs) as the anchors to fix pigments at the appropriate positions through hydrophobic interactions.

The biosynthesis of BChl-*a* in purple bacteria is well understood, and most of the genes encoding biosynthesis enzymes have been identified [5-10]. At the final stage of BChl-*a* biosynthesis, bacteriochlorophyllide(BChlide)-*a*, a precursor possessing a carboxy group at the 17²-position, is esterified with geranylgeranyl (GG) diphosphate by BChl synthase (BchG) to give geranylgeranylated BChl-*a* [11,12]. Successive hydrogenation of three of the four C=C double bonds in GG by GG reductase (BchP) yields mature BChl-*a* esterified with Phy via the intermediates possessing dihydrogeranylgeranyl (DHGG) and tetrahydrogeranylgeranyl (THGG) esters as shown in Fig. 1(b) [13-16]. In the alternative pathway, GG diphosphate is first reduced, and the resulting Phy diphosphate reacts with BChlide-*a* by BChl synthase to give phytylated BChl-*a*. On the other hand, the biosynthesis of photoactive BPhe-*a* is unclear: whether bacteriopheophorbide(BPheide)-*a* (demetalized form of BChlide-*a*) is esterified with GG (Phy) diphosphate or BChlide-*a* is esterified with GG (Phy) diphosphate and then

demetalized.

In our previous study, we isolated LHs and RCs from some *Rhodopseudomonas* (*Rps.*) *palustris* species and analyzed their BChl-*a* and BPhe-*a* in terms of the 17-propionate ester groups [17], since the organisms accumulated BChl-*a* and BPhe-*a* intermediates esterified with GG, DHGG and THGG in a remarkable quantity [18,19]. As a result, the isolated LHs and RCs contained such BChl-*a* and BPhe-*a* intermediates in distinct amounts in addition to the usual Phy esters. These observations clearly demonstrate that BChls-*a* and BPhe-*a* carrying GG, DHGG and THGG esters are not only biosynthetic intermediates but also photosynthetically active pigments in the organisms. In this study, the presence of BChls-*a* and BPhe-*a* esterified with GG, DHGG and THGG in the six phylogenetically distinct classes of purple bacteria (α -1, α -2, α -3, α -4, β and γ *Proteobacteria*) was examined to verify the above findings. For this purpose, we improved high-performance liquid chromatography (HPLC) in order to detect traces of BChls-*a* and BPhe-*a* carrying GG, DHGG and THGG esters. Based on a systematic analysis, we discuss one possible physiological role of the long esterifying substituents in photosynthetic organisms.

2. Material and methods

2.1. Sources and cultivation of purple bacteria

We used fourteen species of purple bacteria containing BChl-*a* and BPhe-*a*. *Rhodospirillum* (*Rsp.*) *rubrum* S1, *Rhodopseudomonas* sp. Rits, *Rhodobacter* (*Rba.*) *sphaeroides* 2.4.1, *Phaeospirillum* (*Phs.*) *molischianum* DSM120 and *Rhodocyclus* (*Rcy.*) *tenuis* DSM109 were grown in PYS medium [20]. *Rhodoferrax* (*Rfx.*) *fermentans*

DSM10138, *Rsp. photometricum* DSM122 and *Allochromatium (Alc.) vinosum* DSM180 were grown in media according to instructions by DSMZ (the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) (DSM medium #26, 27 and 28, respectively). *Rhodovulum (Rdv.) sulfidophilum* DSM1374 and *Rhodomicrobium (Rmi.) vannielii* NBRC10050 were grown in a sodium malate-containing medium as described previously [20]. *Rubrivivax (Rvi.) gelatinosus* NBRC16663 was cultured in the sodium succinate-containing SAY medium as described [20]. *Halorhodospira (Hlr.) halophila* DSM244 was grown in HSE medium as described [21]. *Roseobacter (Rsb.) denitrificans* NBRC15277 and *Erythrobacter (Erb.) longus* NBRC6170 were cultivated in a medium as instructed by NBRC (the National Institute of Technology and Evaluation Biological Resource Center, Chiba, Japan) (NBRC medium #325). *Rsb. denitrificans* and *Erb. longus* were grown under aerobic conditions in the dark for 5 days at 20 and 30 °C, respectively, and the other twelve photosynthetic bacteria were grown anaerobically with a light intensity of ~85 $\mu\text{E}\cdot\text{sec}^{-1}\cdot\text{m}^{-2}$ for 4-6 days at 30 °C.

2.2. Pigment analysis

Pigments (BChl-*a*, BPhe-*a* and some lipophilic components) were extracted from a small amount of full grown cultures of purple bacteria using a mixture of methanol and acetone (1:1, v/v) under sonicated conditions (250 W output and 38 kHz frequency for ~30 sec). The supernatant after centrifugation at 2,000 g (for ~60 sec) was collected. The extraction procedures were repeated three times. The combined extract was evaporated to dryness by a stream of N₂ gas, dissolved in a small amount of 10% (v/v) acetone in methanol, and subjected to the following HPLC. HPLC was performed using

a Shimadzu Prominence liquid chromatograph system comprising a CBM-20A communications bus module, an SPD-M20A photodiode array (PDA) detector, an LC-20AD pump, a DGU-20A₃ degasser and a CTO-20AC column oven (Shimadzu, Kyoto, Japan). Reverse-phase HPLC was performed as follows: column, Cosmosil 2.5C18-MS-II (2.5 μ m, 3.0 x 75 mm, Nacalai Tesque Inc., Kyoto); eluent, 10% (v/v) acetone in methanol; flow rate, 0.40 mL·min⁻¹; range of detection wavelength by PDA, 350-800 nm. We confirmed reproducibility of the data by three independent experiments and evaluated the standard deviation. Liquid chromatography mass spectrometry was performed under the above HPLC conditions, using a Shimadzu LCMS-2010EV system comprising a quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) probe as described [19,22]. The compositions of BChl-*a* and BPhe-*a* esterified with GG, DHGG, THGG and Phy were determined by the peak areas of HPLC monitored at 750 nm. Here we assumed that the four BChl-*a* or four BPhe-*a* had the same molar extinction coefficient values. All solvents used for the analytical HPLC were of HPLC grade quality and were purchased from Nacalai Tesque Inc. and Wako Pure Chemical Industries Ltd. (Osaka, Japan).

2.3. Preparation of authentic BChl-*a* and BPhe-*a* for calibration of HPLC peak areas

Authentic BChl-*a* and BPhe-*a* esterified with Phy were prepared as follows. BChl-*a* was extracted and purified from the harvested cells of *Rba. sphaeroides* 2.4.1 as described [19]. The corresponding BPhe-*a* was prepared from the purified BChl-*a* in methanol (9.0 mL) by addition of 10% aqueous HCl solution (1.0 mL). A mixture of diethyl ether and petroleum ether (1:2, v/v) and then distilled water were added to

transfer the BPhe-*a* component to the ether layer. The resultant BPhe-*a* was used without further purification. The electronic absorption spectra of BChl-*a* and BPhe-*a* thus obtained were recorded in diethyl ether using a Hitachi U-3500 spectrophotometer (Hitachi High-technologies Corp., Tokyo, Japan). Further, BChl-*a* and BPhe-*a* were mixed at an arbitrary molar ratio. The ¹H-nuclear magnetic resonance (NMR) spectrum was recorded in tetrahydrofuran-*d*₈ (Merck KGaA, Darmstadt, Germany) using a JEOL ECA-600 NMR spectrometer (600 MHz, JEOL, Tokyo) as shown in Fig. S1. The residual proton signal of tetrahydrofuran-*d*₇ ($\delta_{(3-H)} = 1.72$ ppm) was used as an internal standard. The relative molar ratio of BChl-*a* to BPhe-*a* in the mixture was determined by integration of their *meso*-proton signals: 5-H (9.05 ppm), 10-H (8.45 ppm) and 20-H (8.37 ppm) for BChl-*a* and 5-H (9.13 ppm), 10-H (8.62 ppm) and 20-H (8.59 ppm) for BPhe-*a*. Simultaneously, the mixture was analyzed by the above mentioned HPLC at 750 nm, and the peak areas of BChl-*a* and BPhe-*a* originating from the absorption (BChl-*a*/BPhe-*a* = 0.483) were corrected using the molar ratio determined by ¹H-NMR (0.484) [23].

3. Results

3.1. HPLC profile and hydrophobicity of BChl-*a* and BPhe-*a* carrying several propionate-type esters

Figure 2(a) shows a representative reverse-phase HPLC profile of the pigments extracted from full grown cultures of *Rhodopseudomonas* sp. Rits. The detection wavelength was set to the Qy-absorption maximum of less abundant BPhe-*a*. Four BChl-*a* (peaks #1-4) and four BPhe-*a* (peaks #5-8) were identified on the basis of their

characteristic electronic absorption spectra as shown in Fig. 2(b). The total ratio of BChl-*a* to BPhe-*a* was determined to be 78.6 ± 5.5 as shown in the last column of Table S1. These BChls-*a* and BPhes-*a* were assigned to the pigments carrying different 17-propionate ester groups: peaks #1/5, GG; #2/6, DHGG; #3/7, THGG; #4/8, Phy. The assignment was confirmed by APCI mass spectrometry, and was consistent with the literature [14,16,19,24]; the details of the characterization are shown in *Supporting Information*.

[Insert Figure 2]

DHGG and THGG groups in the 17-propionate residue are created at the final stage of BChl-*a* biosynthesis by stepwise reduction from a GG to Phy group. Hydrogenation of one or two of the four C=C double bonds in GG gives DHGG or THGG, respectively. The stepwise hydrogenation drastically alters the hydrophobicity and rigidity of the ester groups, thereby regulating the hydrophobicity of BChl-*a* and BPhe-*a* pigments. The hydrophobicity of the esters in the 17-propionate residues was estimated by their calculated hydrophobic parameters, logP, as follows: 5.90, 6.37, 6.85 and 7.32 for geranylgeraniol, dihydrogeranylgeraniol, tetrahydrogeranylgeraniol and phytol, respectively. These values were obtained by ChemDraw Ver. 13.0 software. The elution order of BChls-*a* and BPhes-*a* esterified with such alcohols on reverse-phase HPLC was consistent with the calculated logP, indicating that hydrophobicity of the pigments was drastically modified by the stepwise reduction.

3.2. Compositions of BChl-*a* and BPhe-*a* variants in purple bacteria

Figure 3 and Table S1 summarize the relative compositions (%) of BChls-*a* (to the left in each figure) and BPhes-*a* (to the right) esterified with GG, DHGG, THGG and

Phy found in the six phylogenetically distinct classes of purple bacteria (α -1, α -2, α -3, α -4, β and γ). The compositions were determined by the peak areas of HPLC at 750 nm after calibration of BChl-*a* and BPhe-*a* absorption in HPLC (see 2.3. *Material and methods section*). All the bacteria investigated here (14 species) showed the variations of the 17-propionate ester groups in both BChl-*a* and BPhe-*a*, with the exception of BPhe-*a* in *Rfx. fermentans* (class, β) and *Alc. vinosum* (γ) (see also Table S1). These two bacteria exclusively contained BPhe-*a* with Phy within the detection limit of HPLC. The five bacteria, *Rsp. rubrum* (α -1), *Rsp. photometricum* (α -1), *Rhodopseudomonas* sp. Rits (α -2), *Rmi. vanniellii* (α -2) and *Hlr. halophila* (γ), exhibited significant accumulations of both BChl-*a* and BPhe-*a* intermediates esterified with GG, DHGG and THGG ($\geq 10\%$ of the total BChl-*a* or BPhe-*a* content). In the four bacterial species, *Rsp. rubrum*, *Rsp. photometricum*, *Rhodopseudomonas* sp. Rits and *Hlr. halophila*, drastic decreases of the BPhe-*a* intermediates were observed, compared to the corresponding BChls-*a* (see also the second last column of Table S1). Especially, BPhe-*a* with less reduced and rigid esterifying groups (GG and DHGG) decreased, whereas BPhe-*a* with more reduced and flexible THGG still accumulated to a measurable level. These observations were also seen in the other ten bacterial species, although their compositional changes were small. BPhe-*a* esterified with GG, DHGG and THGG in addition to the dominant Phy was identified for *Rsp. rubrum* at 11.4% of the total BPhe-*a*. In the same organism, it was reported that the esterifying group of BPhe-*a* was exclusively Phy, whereas that of BChl-*a* was GG [25,26].

[Insert Figure 3]

To clarify demetalation of BChl-*a* to produce BPhe-*a* as an artifact during handling of materials, the relative ratios of BChl-*a* to BPhe-*a* in purple bacteria were

evaluated. The ratios were determined by the peak areas of HPLC at 750 nm as shown in the last column of Table S1. The ratios were distributed over a wide range ($17.4 \pm 0.8 \sim 81.0 \pm 2.6$) depending on the organisms, due to the presence or absence of their peripheral LH2 complexes. The ratio in *Rsp. rubrum* which does not contain peripheral LH2 is calculated to be 18:1 in the following manner. The X-ray crystallographic analyses of purple bacterial RCs revealed that the molecular numbers of BChl-*a* and BPhe-*a* were 4:2 in the single RC [27,28]. The LH1 complex of *Rsp. rubrum* surrounding its RC was composed of 16 LH subunits $[(\alpha\beta)_{16}]$ by electron cryomicroscopy [29] and atomic force microscopy [30]; each subunit contained two BChl-*a* molecules. Thus, the ratio of BChl-*a* to BPhe-*a* in *Rsp. rubrum* is calculated to be 18:1 ($= 36 \text{ BChls-}a / 2 \text{ BPhe-}a$). This value was well consistent with that determined by the present HPLC (18.5 ± 1.1), indicating that the demetalation of BChl-*a* to produce BPhe-*a* as an artifact was negligible in the present experiments.

4. Discussion

As mentioned in the Introduction, stepwise reduction of a GG to a Phy group is catalyzed by enzyme BchP at the final stage of BChl-*a* biosynthesis. The *bchP* deletion mutant of *Rba. sphaeroides* showed an electronic absorption spectrum indicating predominance of LH1 over peripheral LH2, compared to the corresponding wild type [14]. This observation is reminiscent of a geranylgeranylated BChl-*a* producing purple bacterium *Rsp. rubrum* which is unable to form LH2. These facts indicate that either

assembly or stability of LH2 is impaired more than that of LH1 by replacement of phytylated BChl-*a* with geranylgeranylated BChl-*a*. In this study, the three bacteria, *Rsp. photometricum*, *Rhodopseudomonas* sp. Rits and *Hlr. halophila* which are able to form LH2, contained geranylgeranylated BChl-*a* in a large amount in addition to phytylated BChl-*a*. Thus, either flexibility of the 17-propionate ester group in BChl-*a* or a mixing of several different esters in BChl-*a* would be crucial for the construction of stable peripheral LH2.

Demetalation of Chls to yield Phe pigments is essential for formation of the primary electron acceptor in photosystem(PS)-II type RCs [27], although the details of the formation are still unclear. In this study, a decrease in the ratio of BPhe-*a* esterified with GG, DHGG and THGG over the total BPhe-*a* content was observed, compared to the corresponding BChls-*a*, especially for the most rigid geranylgeranylated BPhe-*a*; BPhe-*a* with GG was detected at most 10% of the total BPhe-*a* in *Rsp. photometricum*, while BChl-*a* with GG was >40%. Such a decrease was confirmed for all the bacteria investigated here, although the compositional changes were small. This observation indicates that BPhe-*a* localized in RCs is preferentially esterified with two or three fold hydrogenated and flexible chains, i.e. THGG and Phy. The present results obtained from whole cells of purple bacteria were consistent with those from the isolated LHs and RCs of some *Rps. palustris* species (CGA009, Morita and Rits strains) [17,18], indicating that both BChl-*a* and BPhe-*a* esterified with GG, DHGG and THGG in mature cells were not mere biosynthetic intermediates or degradation products but photosynthetically active pigments. To clarify the preference of the esterifying group in BPhe, we analyzed BPhe pigments in the other two types of photosynthetic organisms containing PS-II type RCs: a purple photosynthetic bacterium *Blastochloris viridis* containing BChl-*b*/BPhe-*b*

and a green filamentous anoxygenic phototroph *Chloroflexus aurantiacus* containing BChl-*a*/BPhe-*a*/BChl-*c*. From their HPLC analyses, these organisms exclusively contained phytylated BPhe-*b* or BPhe-*a* within its detection limit (data not shown). Thus, BPhe pigments esterified with more reduced and flexible chains were common in photosynthetic organisms containing PS-II type RCs.

The differences found in the compositions of the 17-propionate ester groups between BChl-*a* and BPhe-*a* suggest the following three possibilities. 1) GG reductase BchP acts after the esterification of GG diphosphate to BChlide-*a* or BPheide-*a* by BchG synthase. In that case, BchP should have substrate specificity dependent on the tetrapyrrole moieties to give different compositions between BChl-*a* and BPhe-*a*. 2) BchP acts before the esterification. In that case, BchG must have different substrate specificity for BChlide-*a* and BPheide-*a*. 3) There is a specific pheophytinization enzyme catalyzing demetalation of a Mg atom in BChl-*a* (magnesium de-chelatase) to yield photoactive phytylated BPhe-*a*. In that case, the enzyme must have substrate specificity depending on the 17-propionate residues.

5. Conclusions

In this study, accumulations of BChl-*a* and BPhe-*a* carrying different 17-propionate ester groups in various purple photosynthetic bacteria were demonstrated using HPLC. The present HPLC enables us to evaluate the presence of the less abundant BPhe-*a* in RCs. All the bacteria investigated here (14 species) showed the variants of

the ester groups derived from the stepwise hydrogenation by BchP in both BChl-*a* and BPhe-*a*, except a few species, although the phylogenetic relationship among the accumulations could not be ruled out. A decrease of BPhes-*a* with GG and DHGG, compared to the corresponding BChls-*a*, indicates that BPhe-*a* molecules in RCs preferably would take more reduced and flexible esters (THGG and Phy). GG is more hydrophilic and less flexible than Phy due to its three additional and unreduced C=C double bonds. This greater rigidity would severely disrupt the packing of the pigment molecules within pigment-protein complexes, especially for BChl-*a* in peripheral LHs and BPhe-*a* in RCs. The reduction level of GG in the 17-propionate residues of Chl molecules would regulate assembly and/or stability of pigment-protein complexes for optimal growth of photosynthetic organisms.

Acknowledgments

This work was partially supported by Grants-in-Aid for Scientific Research (A) (No. 22245030 to HT), (C) (No. 24550065 to TM) and for Young Scientists (B) (No. 24750169 to JH and No. 26840099 to YT) as well as for Scientific Research on Innovative Areas (“Artificial Photosynthesis (AnApple)”, No. 24107002 to HT) from the Japan Society for the Promotion of Science (JSPS). YT is supported by a PRESTO grant from the Japan Science and Technology Agency (JST). MI was supported by a JSPS Fellowship for Young Scientists (No. 10137).

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version,
at <http://dx.doi.org/10.1016/j.jphotobiol.2014>.

References

- [1] H. Scheer, The pigments, in: B.R. Green, W.W. Parson (Eds.), *Light-Harvesting Antennas in Photosynthesis*, Kluwer Academic Publishers, Dordrecht, 2003, pp. 29-81.
- [2] H. Tamiaki, R. Shibata, T. Mizoguchi, The 17-propionate function of (bacterio)chlorophylls: biological implication of their long esterifying chains in photosynthetic systems, *Photochem. Photobiol.* 83 (2007) 152-162.
- [3] T. Mizoguchi, Y. Kimura, T. Yoshitomi, H. Tamiaki, The stereochemistry of chlorophyll-*c*₃ from the haptophyte *Emiliana huxleyi*: the (¹³*R*)-enantiomers of chlorophylls-*c* are exclusively selected as the photosynthetically active pigments in chromophyte algae, *Biochim. Biophys. Acta* 1807 (2011) 1467-1473.
- [4] L. Fiedor, A. Kania, B. Myśliwa-Kurdziel, Ł. Orzeł, G. Stochel, Understanding chlorophylls: central magnesium ion and phytol as structural determinants, *Biochim. Biophys. Acta* 1777 (2008) 1491-1500.
- [5] A.G.M. Chew, D.A. Bryant, Chlorophyll biosynthesis in bacteria: the origins of structural and functional diversity, *Annu. Rev. Microbiol.* 61 (2007) 113-129.
- [6] T. Masuda, Y. Fujita, Regulation and evolution of chlorophyll metabolism, *Photochem. Photobiol. Sci.* 7 (2008) 1131-1149.
- [7] D.W. Bollivar, J.Y. Suzuki, J.T. Beatty, J.M. Dobrowolski, C.E. Bauer, Directed mutational analysis of bacteriochlorophyll *a* biosynthesis in *Rhodobacter capsulatus*, *J. Mol. Biol.* 237 (1994) 622-640.

- [8] Y. Tsukatani, H. Yamamoto, J. Harada, T. Yoshitomi, J. Nomata, M. Kasahara, T. Mizoguchi, Y. Fujita, H. Tamiaki, An unexpectedly branched biosynthetic pathway for bacteriochlorophyll *b* capable of absorbing near-infrared light, *Sci. Rep.* 3 (2013) 1217.
- [9] S.A. Coomber, M. Chaudhri, C.N. Hunter, Localised transposon Tn5 mutagenesis of the photosynthetic gene cluster of *Rhodobacter sphaeroides*, *Mol. Microbiol.* 4 (1990) 977-989.
- [10] G.W. Naylor, H.A. Addlesee, L.C.D. Gibson, C.N. Hunter, The photosynthesis gene cluster of *Rhodobacter sphaeroides*, *Photosynth. Res.* 62 (1999) 121-139.
- [11] U. Oster, C.E. Bauer, W. Rüdiger, Characterization of chlorophyll *a* and bacteriochlorophyll *a* synthases by heterologous expression in *Escherichia coli*, *J. Biol. Chem.* 272 (1997) 9671-9676.
- [12] H.A. Addlesee, L. Fiedor, C.N. Hunter, Physical mapping of *bchG*, *orf427* and *orf177* in the photosynthesis gene cluster of *Rhodobacter sphaeroides*: functional assignment of bacteriochlorophyll synthase gene, *J. Bacteriol.* 182 (2000) 3175-3182.
- [13] W. Rüdiger, The last steps of chlorophyll synthesis, in: K.M. Kadish, K.M. Smith, R. Guilard (Eds.), *The Porphyrin Handbook*, Academic Press, San Diego, 2003, Vol. 13, pp. 71-108.
- [14] H.A. Addlesee, C.N. Hunter, Physical mapping and functional assignment of the geranylgeranyl-bacteriochlorophyll reductase gene, *bchP*, of *Rhodobacter sphaeroides*, *J. Bacteriol.* 181 (1999) 7248-7255.
- [15] H.A. Addlesee, C.N. Hunter, *Rhodospirillum rubrum* possesses a variant of the *bchP* gene, encoding geranylgeranyl-bacteriopheophytin reductase, *J. Bacteriol.*

184 (2002) 1578-1586.

[16] Y. Shioi, T. Sasa, Terminal steps of bacteriochlorophyll *a* phytol formation in purple photosynthetic bacteria, J. Bacteriol. 158 (1984) 340-343.

[17] T. Mizoguchi, M. Isaji, J. Harada, H. Tamiaki, Isolation and pigment composition of the reaction centers from purple photosynthetic bacterium *Rhodopseudomonas palustris* species, Biochim. Biophys. Acta 1817 (2012) 395-400.

[18] J. Harada, T. Mizoguchi, S. Yoshida, M. Isaji, H. Oh-oka, H. Tamiaki, Composition and localization of bacteriochlorophyll *a* intermediates in the purple photosynthetic bacterium *Rhodopseudomonas* sp. Rits, Photosynth. Res. 95 (2008) 213-221.

[19] T. Mizoguchi, J. Harada, H. Tamiaki, Structural determination of dihydro- and tetrahydrogeranylgeranyl groups at the 17-propionate of bacteriochlorophylls-*a*, FEBS Lett. 580 (2006) 6644-6648.

[20] K.V. Nagashima, A. Hiraishi, K. Shimada, K. Matsuura, Horizontal transfer of genes coding for the photosynthetic reaction centers of purple bacteria, J. Mol. Evol. 45 (1997) 131-136.

[21] J.F. Imhoff, Family Ectothiorhodospiraceae, in: D.J. Brenner, N.R. Krieg, J.T. Staley (Eds.), Bergey's Manual of Systematic Bacteriology: The Grammaproteobacteria, Springer, New York, 2005, pp. 40-43.

[22] T. Mizoguchi, J. Harada, H. Tamiaki, Characterization of chlorophyll pigments in the mutant lacking 8-vinyl reductase of green photosynthetic bacterium *Chlorobaculum tepidum*, Bioorg. Med. Chem. 20 (2012) 6803-6810.

[23] R. Fujii, M. Kita, M. Doe, Y. Inuma, N. Oka, Y. Takaesu, T. Taira, M. Iha, T. Mizoguchi, R.J. Cogdell, H. Hashimoto, The pigment stoichiometry in a

chlorophyll *a/c* type photosynthetic antenna, *Photosynth. Res.* 111 (2012) 165-172.

[24] Y. Shioi, R. Fukae, T. Sasa, Chlorophyll analysis by high-performance liquid chromatography, *Biochim. Biophys. Acta* 722 (1983) 72-79.

[25] J.J. Katz, H.H. Strain, A.L. Harkness, M.H. Studier, W.A. Svec, T.R. Janson, B.T. Cope, Esterifying alcohols in the chlorophylls of purple photosynthetic bacteria. A new chlorophyll, bacteriochlorophyll (gg), all-*trans*-geranylgeranyl bacteriochlorophyllide *a*, *J. Am. Chem. Soc.* 94 (1972) 7938-7939.

[26] A. Gloe, N. Pfennig, Occurrence of phytol and geranylgeraniol in the bacteriochlorophylls of red and green sulfur bacteria, *Arch. Microbiol.* 96 (1974) 93-101.

[27] J.P. Allen, J.C. Williams, Reaction centers from purple bacteria, in: P. Fromme (Ed.), *Photosynthetic Protein Complexes: A Structural Approach*, Wiley-VCH Verlag, Weinheim, 2008, pp. 275-293.

[28] A.W. Roszak, K. McKendrick, A.T. Gardiner, I.A. Mitchell, N.W. Isaacs, R.J. Cogdell, H. Hashimoto, H.A. Frank, Protein regulation of carotenoid binding: gatekeeper and locking amino acid residues in reaction centers of *Rhodobacter sphaeroides*, *Structure* 12 (2004) 765-773.

[29] S.J. Jamieson, P. Wang, P. Qian, J.Y. Kirkland, M.J. Conroy, C.N. Hunter, P.A. Bullough, Projection structure of the photosynthetic reaction centre-antenna complex of *Rhodospirillum rubrum* at 8.5 Å resolution, *EMBO J.* 21 (2002) 3927-3935.

[30] D. Fotiadis, P. Qian, A. Philippsen, P.A. Bullough, A. Engel, C.N. Hunter, Structural analysis of the RC-LH1 photosynthetic core complex of

440 *Rhodospirillum rubrum* using atomic force microscopy, J. Biol. Chem. 279 (2004)

441 2063-2068.

442

443

Figure captions

Figure 1. (a) Molecular structures of BChl-*a* (M = Mg) and BPhe-*a* (M = 2H) in purple photosynthetic bacteria. (b) Variations of the ester groups in the 17-propionate residues of BChl-*a* and BPhe-*a* found in purple photosynthetic bacteria: geranylgeranyl (GG), dihydrogeranylgeranyl (DHGG), tetrahydrogeranylgeranyl (THGG) and phytyl (Phy) (from top to bottom). XOH represents BChlide-*a* (M = Mg), BPheide-*a* (M = 2H) or diphosphate.

Figure 2. (a) The representative reverse-phase HPLC profile monitored at 750 nm of the pigments extracted from full grown cultures of *Rhodopseudomonas* sp. Rits. The inset panel shows expansion of the chromatogram at the retention times of 5-10 min ($\times 25$). #1: BChl-*a* with GG; #2: BChl-*a* with DHGG; #3: BChl-*a* with THGG; #4: BChl-*a* with Phy; #5: BPhe-*a* with GG; #6: BPhe-*a* with DHGG; #7: BPhe-*a* with THGG; #8: BPhe-*a* with Phy. Degradation and unidentified components are indicated by asterisks. (b) Electronic absorption spectra of authentic phytolated BChl-*a* (solid) and BPhe-*a* (dotted) isolated from *Rba. sphaeroides* 2.4.1 in diethyl ether: normalized at intense Soret maxima. Absorption maxima of BPhe-*a* are shown in parenthesis.

Figure 3. The relative composition (%) of BChls-*a* (each left) and BPhes-*a* (each right) carrying GG (■), DHGG (▤), THGG (▥) and Phy groups (□) in the 17-propionate residue determined by peak areas of HPLC at 750 nm. The vertical scale expands to 50% to clarify traces of components. In the left column of *Rsp. rubrum*, the relative composition of BChl-*a* with GG is 98.9%. The original data with its standard deviation is summarized in Table S1.

Figure 1
Mizoguchi et al.

Figure 2
Mizoguchi et al.

Figure 3
Mizoguchi et al.