Biochimica et Biophysica Acta 1817 (2012) 395–400

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

Isolation and pigment composition of the reaction centers from purple photosynthetic bacterium *Rhodopseudomonas palustris* species

Tadashi Mizoguchi [a], Megumi Isaji [a], Jiro Harada [b], Hitoshi Tamiaki [a,*]

[a] Department of Bioscience and Biotechnology, Faculty of Science and Engineering, Ritsumeikan University, Kusatsu, Shiga 525–8577, Japan
[b] Department of Medical Biochemistry, Kurume University School of Medicine, Kurume, Fukuoka 830–0011, Japan

ARTICLE INFO

Article history:
Received 7 October 2011
Received in revised form 2 December 2011
Accepted 5 December 2011
Available online 13 December 2011

Keywords:
Reaction center
Rhodopseudomonas palustris
Bacteriochlorophyll-*a*
Bacteriopheophytin-*a*
17-Propionate

ABSTRACT

The reaction centers (RCs) from several species of a purple photosynthetic bacterium, *Rhodopseudomonas palustris*, were first isolated by ammonium-sulfate fractionation of the isolated core complexes, and were successfully purified by anion-exchange and gel-filtration chromatography as well as sucrose-density gradient centrifugation. The RCs were characterized by spectroscopic and biochemical analyses, indicating that they were sufficiently pure and had conserved their redox activity. The pigment composition of the purified RCs was carefully analyzed by LCMS. Significant accumulation of both bacteriochlorophyll(BChl)-*a* and bacteriopheophytin(BPhe)-*a* esterified with various isoprenoid alcohols in the 17-propionate groups was shown in RCs for the first time. Moreover, a drastic decrease in BPhe-*a* with the most dehydrogenated and rigid geranylgeranyl(GG) ester was observed, indicating that BPhe-*a* in RC preferably took partially hydrogenated and flexible ester groups, i.e. dihydro-GG and tetrahydro-GG in addition to phytyl. Based on the reported X-ray crystal structures of purple bacterial RCs, the meaning of flexibility of the ester groups in BChl-*a* and BPhe-*a* as the cofactors of RCs is proposed.

1. Introduction

All naturally occurring (bacterio)chlorophyll [(B)Chl] pigments have a propionate-type ester group at the 17^2-position, with the exception of most Chls-*c* which have a free acrylic residue at the 17-position [1–3]. In general, Chl-*a* found in oxygenic phototrophs and BChl-*a* [see Fig. 1(a), M=Mg] in anoxygenic photosynthetic bacteria have a C_{20} phytyl group as their ester group R in Fig. 1(a) [1,2]. The esterifying substituent is not directly conjugated with the π-system in (B)Chl molecules and does not affect the electronic absorption spectra of their monomeric states (removal of the phytyl to form the corresponding free carboxylic acid, however, induced small but meaningful effects [4]). As a result, the moiety has attracted less attention than other peripheral substituents, although it constitutes about 30% of the weight of a molecule [1,2], and this huge substituent serves as an anchor to lock (B)Chl molecules in the appropriate positions in pigment-protein complexes through hydrophobic interactions.

At the final stage of (B)Chl biosynthesis, except for most Chls-*c*, (bacterio)chlorophyllides which have a carboxy group at the 17^2-position are esterified with various long hydrocarbon-chains [5]. In anoxygenic photosynthetic bacteria [6,7], bacteriochlorophyllide-*a* [R=H in Fig. 1(a)] was esterified with geranylgeranyl (GG) diphosphate by a BChl synthase (BchG) to give geranylgeranylated BChl-*a*, and successive hydrogenation of three of its four C=C double bonds by a GG reductase (BchP) yielded phytylated BChl-*a* via the intermediates with dihydrogeranylgeranyl (DHGG) and tetrahydrogeranylgeranyl (THGG) esters [see Fig. 1(b)]. In the alternative pathway, GG diphosphate is first reduced and the resulting phytyl (Phy) diphosphate reacts with bacteriochlorophyllide-*a* in a reaction catalyzed by BchG to give mature BChl-*a* with Phy. The actual biosynthetic pathway has not yet been determined and it is not known whether the hydrogenation occurs before or after esterification, or both.

From some batch cultures of purple photosynthetic bacteria, BChls-*a* esterified with a GG, DHGG or THGG group in addition to mature BChl-*a* with Phy were detected in at most 4% of the total BChl-*a* component by HPLC [6–8] until our previous report [9]. We found significant accumulation of such BChls-*a* with various 17-propionates in several purple bacteria (at most, half of the total BChl-*a*), especially for *Rhodopseudomonas* (*Rps.*) *palustris* species that biosynthesized unusual peripheral antennae termed light-harvesting (LH) complexes 4 instead of common LH2 under low-light illuminated conditions [9–11]. Using these organisms, we could isolate a large quantity of pure BChls-*a* with a DHGG or THGG substituent and determine their molecular structures, positions of C=C double bonds in the ester

Abbreviations: APCI, atmospheric pressure chemical ionization; (B)Chl, (bacterio)chlorophyll; *Blc.*, *Blastochloris*; BPhe, bacteriopheophytin; DHGG, dihydrogeranylgeranyl; GG, geranylgeranyl; LH, light-harvesting; LDAO, *N,N*-dimethyldodecylamine-*N*-oxide; OD, optical density; Phy, phytyl; *Rba.*, *Rhodobacter*; RC, reaction center; *Rps.*, *Rhodopseudomonas*; *Rsp.*, *Rhodospirillum*; THGG, tetrahydrogeranylgeranyl; Tris, 2-amino-2-(hydroxymethyl)-aminomethane-1,3-propanediol.

* Corresponding author. Tel.: +81 77 561 2765; fax: +81 77 561 2659.
E-mail address: tamiaki@se.ritsumei.ac.jp (H. Tamiaki).

0005-2728/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbabio.2011.12.001

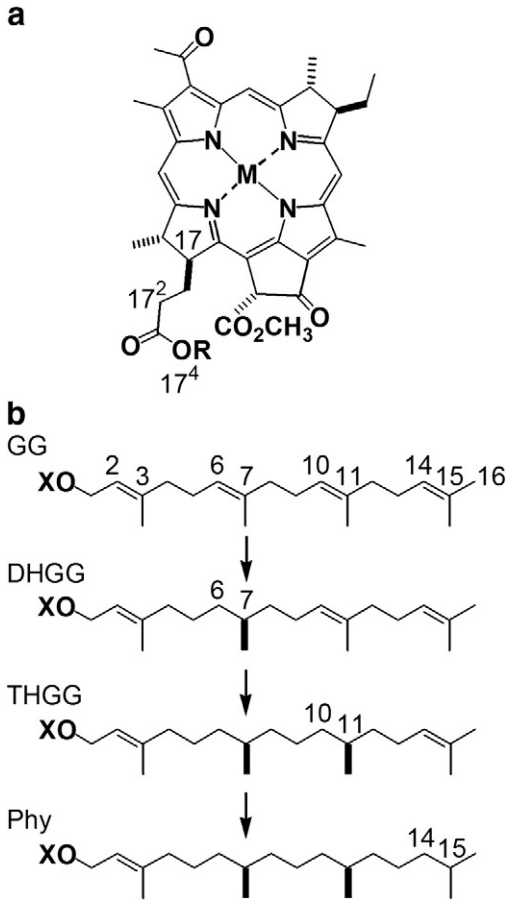

Fig. 1. Molecular structures of BChl-*a* (M=Mg) and BPhe-*a* (M=H_2) (a) and stepwise hydrogenation of geranylgeranyl (GG) to phytyl (Phy) groups with the abbreviations of ester groups (b): XOR=BChl-*a* or diphosphate.

groups, without ambiguity: the DHGG=Δ2,10,14-phytatrienyl and the THGG=Δ2,14-phytadienyl [12]. Based on the precisely determined structures of the ester groups in BChl-*a*, we proposed the sequential hydrogenation of its GG to Phy group at C6=C7→C10=C11→C14=C15 as shown in Fig. 1(b) [12]. In addition to the foregoing sequence, other (B)Chls with different C_{20} propionate ester groups were found in some photosynthetic bacteria [1,2]. For example, Steiner et al. reported the presence of another THGG ester group (Δ2,10-phytadienyl) in BChl-*b* as the dominant light-harvesting, energy-migrating and electron-transferring BChl pigments from a thermophilic purple bacterium, *Halorhodospira halochloris* (previously named *Ectothiorhodospira halochloris*) [13]. Moreover, Kobayashi et al. reported the presence of the other THGG ester group (Δ2,6-phytadienyl) in Chl-*a* as a primary electron-accepting Chl pigment in the reaction center (RC) of a thermophilic green bacterium, *Chlorobaculum tepidum* (previously *Chlorobium tepidum*) [14]. These two exceptions are found in thermophilic bacteria, indicating that the structures of the 17-propionate ester group in (B)Chl molecules would play an important role in constructing photosynthetic apparatuses.

In our previous studies on accumulation and distribution of such BChl-*a* intermediates in photosynthetic apparatuses, they were preferentially found in core complexes [RC-LH1 complexes] over the peripheral antennae (LH2 and/or LH4) [9]. The distribution in RCs prepared from *Rps. palustris* species has not been evaluated, since these were not isolated. This is probably due to the higher stability of core complexes of the organisms than that of the corresponding RCs, which is consistent with the fact that the core complex from *Rps. palustris* alone (strain 2.6.1, often referred to as strain FRENCH) was crystallized and its structure was determined by X-ray analysis [15]. To clarify the distribution of BChl-*a* intermediates in RCs, as well as that of the electron-accepting bacteriopheophytin(BPhe)-*a* [M=H_2 in Fig. 1(a)] only in RCs, we attempted to isolate the RCs from several *Rps. palustris* species and analyzed their pigment composition by LCMS.

2. Materials and methods

2.1. Isolation and purification of RCs

Two strains of *Rps. palustris*, Morita (NBRC 100246) and CGA009, *Rhodopseudomonas* sp. Rits which is phylogenetically close to *Rps. palustris* (our culture collection [9]) and *Rhodobacter* (*Rba.*) *sphaeroides* 2.4.1 were grown anaerobically in the PYS medium with a light intensity of ~85 μE s^{-1} m^{-2} at 30 °C for 4–6 days as described [9]. Under these culturing conditions, all four bacteria biosynthesized usual LH2 as their dominant peripheral antennae (Morita, CGA009 and Rits strains dominantly biosynthesized unusual peripheral LH4 under low-light illuminated conditions).

The core complexes of the aforementioned purple bacteria were obtained by solubilization of photosynthetic membranes with 1% (v/v) *N*,*N*-dimethyldodecylamine-*N*-oxide (LDAO, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) as described [9] (details are shown in Appendix A).

To isolate RCs, we applied ammonium-sulfate fractionation to the core complexes based on the reported method [16]. Briefly, the core complexes after sucrose-density gradient centrifugation [OD_{860} = 50 in 20 mM 2-amino-2-(hydroxymethyl)aminomethane-1,3-propanediol(Tris)–HCl (pH 8.0)] were treated with 2.3 M ammonium sulfate for 1 h at 27 °C in the dark. After centrifugation at 1800 *g* (for 1–2 min at room temperature), the floating pellet was collected by filtration and was resuspended in 20 mM Tris–HCl buffer containing 0.1% LDAO (buffer A). The crude RCs thus obtained were concentrated 10 times by a centrifugal filter device (Amicon Ultra 50 K, Millipore Corp., MA, USA) and were diluted 10 times with buffer A. The concentration and dilution were repeated twice. The finally concentrated RCs were purified using anion-exchange chromatography [DE52(diethylaminoethyl cellulose); GE Healthcare Bio-Science, Tokyo, Japan] equilibrated with buffer A. They were then applied to the column and sufficiently washed with buffer A. To remove free pigments and denatured LH1 subunits, buffer A containing 30 mM NaCl was used. The RC component was eluted with buffer A containing 50 mM NaCl. The RC fraction [absorbance at 280 nm divided by that at 800 nm (A_{280}/A_{800}) < 1.2] was collected and dialyzed against buffer A. Further purification was performed by sucrose-density gradient centrifugation (0, 0.3, 0.6 and 1.2 M sucrose in buffer A, 250,000 *g* at 4 °C for 18 h) and gel-filtration chromatography [Äkta prime plus equipped with a HiPrep Sephacryl S-200HR (16×600 mm) as a column, GE Healthcare Bio-Science] with buffer A containing 150 mM NaCl, and then dialyzed against buffer A and concentrated by the centrifugal filter device to OD_{800} = 5–20.

2.2. Pigment analyses

The pigments (BChl-*a*, BPhe-*a* and some other lipophilic components) were extracted by sonication (250 W output and 38 kHz frequency for 30 s) from the concentrated RC thus purified (OD_{800} = 5–20, 10 μL) using a mixture of methanol (30 μL) and acetone (20 μL). The supernatant after centrifugation at 2000 *g* (for 30–60 s at room temperature) was subjected to the following HPLC: column, Cosmosil 5C18-AR-II (4.6×150 mm) (Nacalai Tesque, Kyoto, Japan); eluent, 10% (v/v) acetone in methanol; flow rate, 1.0 mL min^{-1}; detection wavelength, 750 nm. The compositions of BChl-*a* and BPhe-*a* with different 17-propionate ester groups and

the BChl-*a*/BPhe-*a* ratio were determined by the peak areas of the chromatograms at 750 nm. Here, we assumed the same molar extinction coefficient values of the set of BChl-*a* or BPhe-*a* intermediates: 65.3 $mM^{-1}\,cm^{-1}$ for BChl-*a* and 45.1 $mM^{-1}\,cm^{-1}$ for BPhe-*a* at their near-infrared maxima in a mixture of acetone and methanol [16]. We confirmed the reproducibility of the compositions of BChl-*a* and BPhe-*a* by three independent preparations of RCs. LCMS was performed using a Shimadzu LCMS-2010EV system (Shimadzu, Kyoto) equipped with an atmospheric pressure chemical ionization (APCI) probe as described [12].

2.3. Spectrometry

Electronic absorption spectra of the purified RCs were recorded at room temperature using a Hitachi U-3500 spectrophotometer (Hitachi, Ltd., Tokyo) in buffer A. Stock solutions of potassium ferricianide and sodium ascorbate at a concentration of 500 mM each were prepared in buffer A. By adding each stock solution to the RC suspensions ($A_{802} = 0.4$ in buffer A), chemically oxidized and reduced RC spectra were recorded. All solvents and chemicals were commercially available and were used without further purification.

2.4. Analysis of X-ray crystal structures of purple bacterial RCs

The reported X-ray crystal structures of purple bacterial RCs were investigated using a PyMol software (version 0.99rc6). The data of 48 crystal structures for *Rba. sphaeroides* and 14 structures for *Blastochloris* (*Blc.*) *viridis* were taken from the Protein Data Bank and analyzed in terms of the conformations and fluctuations of the isoprenoid-type long hydrocarbons in BChl-*a* and BPhe-*a* as well as quinonoid and carotenoid molecules.

3. Results and discussion

3.1. Isolation and purification of RCs from Rps. palustris species

The methods for isolation of RCs from various purple photosynthetic bacteria are well established [17], although that for *Rps. palustris* species has not been reported. This would be ascribable to the fact that most researchers who study purple bacteria have used a rather restricted number of species, *Rba. sphaeroides*, *Rba. capsulatus*, *Rhodospirillum* (*Rsp.*) *rubrum*, *Blc. viridis* and *Rps. acidophila*. These were (historically) easily grown and also genetically amenable [18]. The established methods were mainly based on stepwise solubilization of photosynthetic membranes with several kinds of detergents. Using such methods, we tried to isolate the RCs from *Rps. palustris* species, but found them to be less effective for this species. As the X-ray crystallographic structure of the core complex has been obtained only from *Rps. palustris* species [15], these unsuccessful attempts were probably due to the higher stability of the core complex of this species than those of the other purple bacteria. Another procedure based on ammonium-sulfate fractionation [16,17] was applied to isolate the RCs from *Rps. palustris* species, and was found to be effective. Further purification using anion-exchange and gel-filtration chromatography was also useful.

Fig. 2 shows the gel-filtration chromatographic profiles monitored at 280 nm of the RCs after sucrose-density gradient centrifugation (see Section 2.1): *Rhodopseudomonas* sp. Rits, *Rps. palustris* Morita, *Rps. palustris* CGA009 and *Rba. sphaeroides* 2.4.1 are shown from top to bottom. The arrow at the retention time of ~135 min in the profile indicates the elution time of the contaminant core complex. This was confirmed by electronic absorption properties of the RC and core complex as well as their co-chromatographic analysis (see Fig. S1). Asterisks in the profiles indicate denatured components during the handling of materials based on their absorption properties. Using these different elution times of the RC, corresponding core complex and denatured components, we found to isolate the pure RCs from *Rps. palustris* species as their detergent-solubilized forms. The lack of any contaminant of peptides originating from LH components was also confirmed by SDS-PAGE analyses of denatured RCs (see Fig. S2). The RCs of Rits, Morita and CGA009 strains gave slightly longer retention times on the present gel-filtration chromatography than that of *Rba. sphaeroides*, indicating that the three RCs were smaller in size than that of *Rba. sphaeroides*. This is consistent with the calculated molecular weights of the composite peptides, L, M and H: 31.3, 34.4 and 28.1 kDa for L-, M- and H-peptides, respectively, of *Rba. sphaeroides* R-26.1 [19] and 30.9, 34.4 and 27.4 for the corresponding peptides of *Rps. palustris* CGA009 (calculated from its amino acids sequence in the Gene Data Bank).

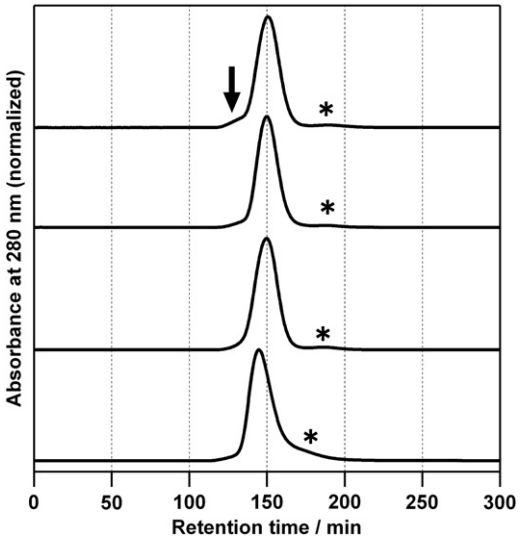

Fig. 2. Gel-filtration chromatograms of the RCs from *Rhodopseudomonas* sp. Rits, *Rps. palustris* Morita, *Rps. palustris* CGA009 and *Rba. sphaeroides* 2.4.1 from top to bottom. Arrow indicates the retention time of the core complex (see the co-chromatography of the isolated RC and core complex from Morita strain in Fig. S1).

3.2. Electronic absorption spectra of RCs

Fig. 3 shows electronic absorption spectra of the purified RCs from *Rhodopseudomonas* sp. Rits, *Rps. palustris* Morita, *Rps. palustris* CGA009 and well-studied *Rba. sphaeroides* 2.4.1 (from top to bottom). The newly isolated RCs from *Rps. palustris* species including Rits strain exhibited the characteristic features of purple bacterial RCs: the Qy bands of special pair BChl-*a* at 872–874 nm, accessory BChl-*a* at 801–802 nm, primary electron acceptor BPhe-*a* at 750–751 nm and the Qx band of special pair BChl-*a* at 596 nm (the amount of BPhe-*a* was not evaluated from its Qx band at around 535 nm where the absorption of more red-shifted carotenoid molecules in *Rps. palustris* species [20] was overlapped). These spectral features are compatible with those of the well-characterized RC from *Rba. sphaeroides* as shown at the bottom of the figure, except for the slightly shifted Qy absorption bands and the bands originating from carotenoids having different numbers of conjugated C=C double bonds (450–550 nm). The absorption ratio at 800 (originating from accessory BChl-*a*) and 280 nm (from aromatic amino acids in peptides), A_{280}/A_{800}, is one of the reliable criteria by which the purity of isolated RCs can be confirmed [17]. The ratios were determined to be 1.10, 1.10, 1.10 and 1.27 for *Rhodopseudomonas* sp. Rits, *Rps. palustris* Morita, *Rps. palustris* CGA009 and *Rba. sphaeroides* 2.4.1, respectively. These values are compatible with those reported previously: typically, 1.22 for *Rba. sphaeroides* [17] and 1.1 for *Rhodobium marinum* [21]. Based on this

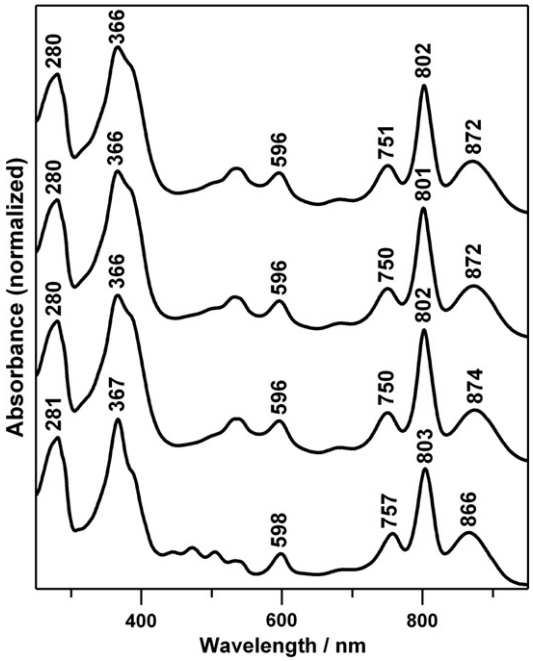

Fig. 3. Electronic absorption spectra of the purified RCs (after gel-filtration chromatographic fractionation) from *Rhodopseudomonas* sp. Rits, *Rps. palustris* Morita, *Rps. palustris* CGA009 and well-studied *Rba. sphaeroides* 2.4.1 (from top to bottom) in 20 mM Tris–HCl buffer (pH 8.0) containing 0.1% (v/v) LDAO.

criterion as well as the similarity of characteristic absorption features, the present isolated RCs were confirmed to be spectroscopically pure.

3.3. Redox activity of RCs

Fig. 4(a) shows the representative electronic absorption spectra of the RC from *Rhodopseudomonas* sp. Rits chemically prepared in the reduced (upper) and the oxidized (lower) forms (produced by the addition of ascorbate and ferricyanide, respectively). The difference spectrum of the oxidized minus the reduced forms is shown in Fig. 4(b). The bleaching of the 871 and 596 nm absorption of the BChl-*a* special pair as well as the blue shift of the 802 nm absorption of the accessory BChl-*a* produce a difference spectrum which is very similar to that seen in other purple bacterial RCs [21,22]. These observations supported that the present isolated RCs conserved their redox activity.

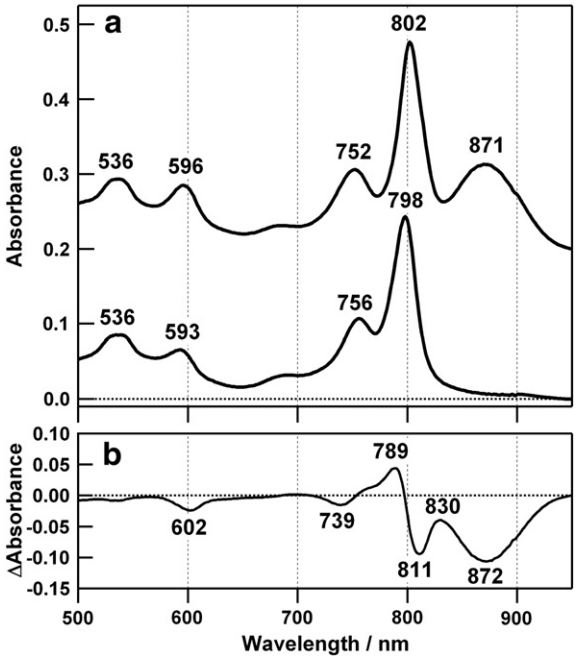

Fig. 4. Redox-dependent electronic absorption spectra of the purified RC from *Rhodopseudomonas* sp. Rits (a): reduction with ascorbate (upper) and oxidation with ferricyanide (lower). The difference spectrum of oxidized minus reduced spectra is shown in panel (b).

3.4. Composition of BChl-a and BPhe-a with various 17-propionates in RCs

Fig. 5(a) shows the representative reverse-phase HPLC profile of the pigment extracts from the purified RC of *Rhodopseudomonas* sp. Rits. Two pairs of chlorophyllous pigments, BChl-*a* and BPhe-*a*, were clearly observed: peaks #1–#4 for BChl-*a* and peaks #1′–#4′ for BPhe-*a*. These pairs of pigments were unambiguously assigned to the pigments having different ester groups at the 17^2-position as shown in Fig. 1(b) by on-line APCI-LCMS: peak #1(1′), GG; peak #2(2′), DHGG (= Δ2,10,14-phytatrienyl); peak #3(3′), THGG (= Δ2,14-phytadienyl); peak #4(4′),

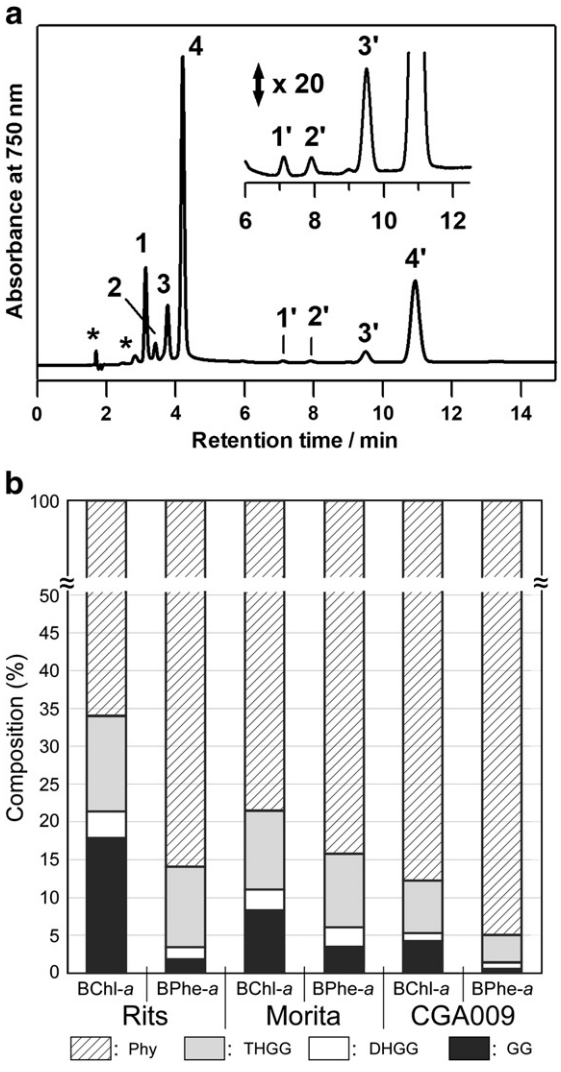

Fig. 5. Representative HPLC profile of the extract from the purified RC of *Rhodopseudomonas* sp. Rits (a) and the composition (%) of BChl-*a* (each left column) and BPhe-*a* (each right column) in the RCs of *Rhodopseudomonas* sp. Rits, *Rps. palustris* Morita and *Rps. palustris* CGA009 (b, left to right columns). Inset in panel (a) corresponds to the expansion of the BPhe-*a* eluted region. Asterisks in panel (a) indicate impurities during handling of materials.

Phy. The assignment of each peak was consistent with the literature, including our previous $^{1}H/^{13}C$ NMR studies of BChl-*a* from Rits strain [6–8,12]. The positions of the two or three C=C double bonds in THGG or DHGG ester groups of BPhe-*a* were confirmed to be the same as those of the corresponding BChls-*a* by the co-chromatographic analysis of BPhe-*a* with the demetallated BChls-*a* (data not shown), since BChl-*a* and BPhe-*a* molecules with isomeric 17-propionate ester groups gave the different retention times on HPLC chromatography [23]. The relative ratio of the total BChl-*a* (peaks #1–#4) to BPhe-*a* (peaks #1′–#4′) was determined to be 2.2 (±0.1) using the peak areas of the chromatogram. This is consistent with the pigment stoichiometry of purple bacterial RCs determined by X-ray, BChl-*a*/BPhe-*a* = 2/1 [19,24], supporting that almost no demetallation of BChl-*a* to yield BPhe-*a* occurred during the present experimental procedures. In addition to mature Phy ester, the intermediates with a GG, DHGG and THGG group of both BChl-*a* and BPhe-*a* were observed in the purified RCs: 34% for BChl-*a* and 14% for BPhe-*a* in Rits strain. These observations reveal that such intermediates can act as photoactive pigments in photosynthesis.

Fig. 5(b) summarizes the relative content (%) of the intermediates in the RCs from *Rhodopseudomonas* sp. Rits, *Rps. palustris* Morita and *Rps. palustris* CGA009 (from left to right columns). Interestingly, the content (%) of the intermediates of BPhe-*a* decreased in comparison with that of BChl-*a* for the three strains investigated here. In BPhe-*a*, the amount of the most dehydrogenated and rigid GG drastically decreased in the three strains, although the decrease in amounts of the other partially hydrogenated DHGG and THGG was relatively small. The results are reminiscent of the following previous findings. The Phy ester of BPhe-*a* was exclusively observed in the RC of *Rsp. rubrum* that biosynthesized GG ester as the dominant BChl-*a* [16] and sole, unique and isomeric THGG (=Δ2,10-phytadienyl) ester in BPhe-*b* was found from *Halorhodospira halochloris* that biosynthesized not Phy but THGG ester in BChl-*b* [13,23]. In RC, discrimination of BPhe-*a* for less hydrogenated ester groups as the 17-propionates is expected.

In our previous report, preferential accumulation of the BChl-*a* intermediates from *Rps. palustris* species was demonstrated in core (RC-LH1) complexes, but not in peripheral antennae (LH2 and/or LH4) [9]. This indicates that BChl-*a* molecules with more flexible ester groups, i.e. phytyl, are preferable for construction of peripheral LH2 and/or LH4 and that core complexes can accommodate BChl-*a* with relatively rigid esters. This is consistent with the following facts. In the purple bacterium *Rsp. rubrum*, BChl-*a* with GG functioned as an alternative of BChl-*a* with Phy, and this species did not produce peripheral antennae (LH2) due to unstable complexation of LH2 oligopeptides with geranylgeranylated BChl-*a* and/or poor assembly of the resulting complexes [25]. Furthermore, in the previous study on the assembly of photosynthetic complexes with geranylgeranylated BChl-*a* in *Rba. sphaeroides*, the mutant lacking *bchP* (encoding GG reductase)

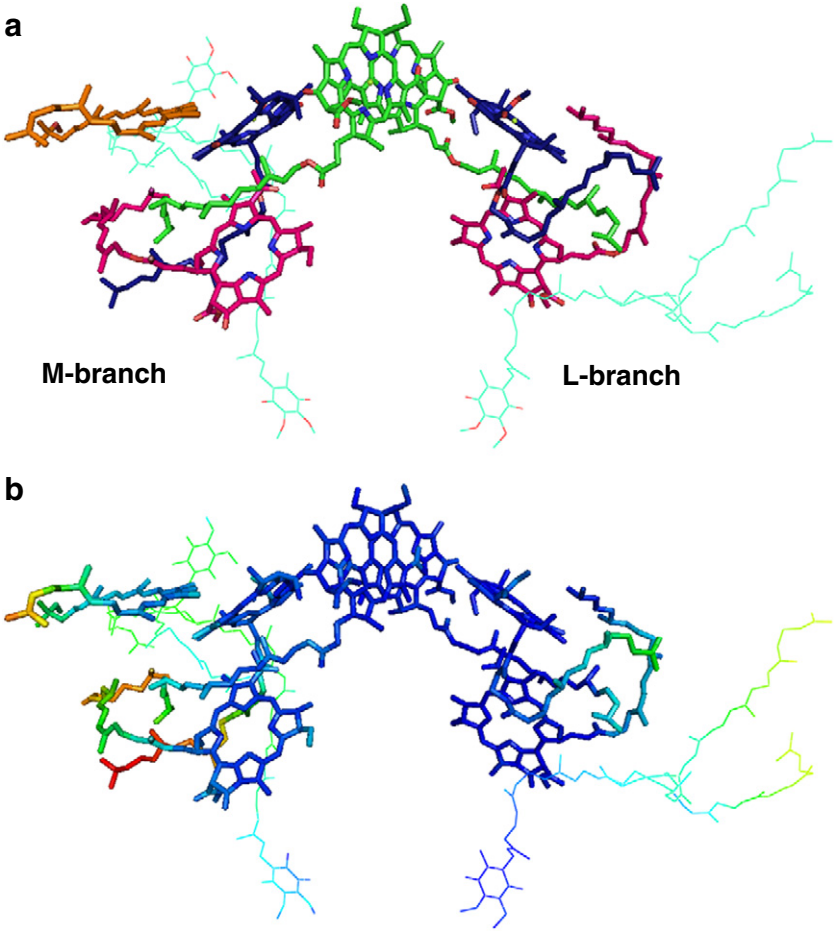

Fig. 6. The crystal structure of the RC from *Rba. sphaeroides* 2.4.1 (PDB code 3I4D) drawn by a PyMol software: (a) Cofactors are differentiated by colors of their composite carbons, special pair BChl-*a* (green), accessory BChl-*a* (blue), BPhe-*a* (magenta), carotenoid (orange) and quinone (light blue with thin line); (b) the structure depicted by B-factor in the X-ray analysis, corresponding to increase with fluctuations of atoms, from blue to red through light blue, green, yellow and orange.

showed a predominance of LH1 over LH2, indicating that the assembly or the stability of LH2 was impaired more than that of LH1 [6].

To evaluate the present significant accumulation of the intermediates of both BChl-*a* and BPhe-*a* in RCs from *Rps. palustris* species, we investigated the reported X-ray structures of RCs from *Rba. sphaeroides* (48 crystal structures) and *Blc. viridis* (14 crystal structures) in terms of the conformations and fluctuations (B-factor in X-ray analysis) of the 17-propionate ester groups of BChl and BPhe molecules, using the data from Protein Data Bank. Fig. 6(a) depicts the representative X-ray crystal structure of the RC from *Rba. sphaeroides* 2.4.1 (PDB code 3I4D). We found the following two structural features. (1) The conformations of the 17-propionates of BChl-*a*/*b* and BPhe-*a*/*b* molecules in the active electron-transferring pathway including the L-branch were apparently confirmed, whereas some of them in the M-branch were invisible (17 of 62 crystal structures), especially for the RCs from *Rba. sphaeroides*. It is noted that these unidentified (fluctuated) structures all are converged in the cofactors of the M-branch, i.e. accessory BChl-*a* and BPhe-*a* as well as quinone (ubiquinone) possessing a long hydrocarbon chain [see Fig. 6(b)]. (2) The well conserved ester groups of accessory BChl-*a*/*b* and BPhe-*a*/*b* in the L-branch were largely twisted, while those of special pair BChls-*a*/*b* were not. Especially, the 17-propionates of BPhe-*a*/*b* were twisted at around the central part of the ester chains.

The aforementioned former feature (1) indicates that recognition of the precise structures of the 17-propionates of accessory BChl-*a*/*b* and BPhe-*a*/*b* in the M-branch might be less strict than those in the L-branch. Their low electron densities of the X-ray diffraction [see also Fig. 6(b)] are probably due to the fluctuation and/or variety of the ester groups as well as to the overlap of the long hydrocarbon chains of BChl and BPhe with that of quinone (ubiquinone for *Rba. sphaeroides* and ubiquinone and menaquinone for *Blc. viridis*). From the foregoing consideration, the intermediates of BChl-*a* and BPhe-*a* with different degree of hydrogenation (flexibility) of the ester groups are potentially applicable for binding to RC as such cofactors in the M-branch. Moreover, from the latter feature (2), exclusion of the rigid ester groups of BChl-*a* and BPhe-*a* in the L-branch is most likely, since the 17-propionate having the tri-substituted olefine at the central part (the presence of the C6=C7 double bond) is difficult to take this unique and twisted conformation.

In the present investigation, we demonstrated that first preparation of the pure RCs from *Rps. palustris* species and significant accumulation of both BChl-*a* and BPhe-*a* esterified with various isoprenoid alcohols in the 17-propionates as photoactive pigments. The preparation is useful for obtaining a set of photosynthetic apparatuses, i.e. usual LH2, low-light adapted LH4, RC-LH1 (core complex) and RC. Using these organisms, the effect of flexibility (site-selective hydrogenation) of the 17-propionate ester group (as well as that of quinonoid and carotenoid molecules) upon construction and/or regulation of photosynthetic apparatuses would be evaluated.

Acknowledgements

This work was partially supported by Grants-in-Aid for Scientific Research (A) (No. 22245030) (to HT) from the Japanese Society for the Promotion of Science (JSPS), and for Young Scientists (B) (No. 22750017) (to TM) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government. MI was supported by a JSPS Fellowship for Young Scientists (No. 10137).

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.bbabio.2011.12.001.

References

[1] 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.
[2] H. Scheer, The pigments, in: B.R. Green, W.W. Parson (Eds.), Light-Harvesting Antennas in Photosynthesis, Kluwer Academic Publishers, Dordrecht, The Netherlands, 2003, pp. 29–81.
[3] T. Mizoguchi, Y. Kimura, T. Yoshitomi, H. Tamiaki, The stereochemistry of chlorophyll-c_3 from the haptophyte *Emiliania huxleyi*: the (13^2R)-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 phytyl as structural determinants, Biochim. Biophys. Acta 1777 (2008) 1491–1500.
[5] W. Rüdiger, The last steps of chlorophyll synthesis, in: K.M. Kadish, K.M. Smith, R. Guilard (Eds.), The Porphyrin Handbook, vol. 13, Academic Press, San Diego, CA, 2003, pp. 71–108.
[6] 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.
[7] Y. Shioi, T. Sasa, Terminal steps of bacteriochlorophyll *a* phytol formation in purple photosynthetic bacteria, J. Bacteriol. 158 (1984) 340–343.
[8] C. Francke, J. Amesz, The size of photosynthetic unit in purple bacteria, Photosynth. Res. 46 (1995) 347–352.
[9] 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.
[10] R.J. Cogdell, A. Gall, J. Köhler, The architecture and function of the light-harvesting apparatus of purple bacteria: from single molecules to *in vivo* membranes, Quarterly Rev. Biophys. 39 (2006) 227–324.
[11] H. Hayashi, M. Miyao, S. Morita, Absorption and fluorescence spectra of light-harvesting bacteriochlorophyll-protein complexes from *Rhodopseudomonas palustris* in the near-infrared region, J. Biochem. 91 (1982) 1017–1027.
[12] 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.
[13] R. Steiner, W. Schäfer, I. Blos, H. Wieschhoff, H. Scheer, Δ2,10-Phytadienol as esterifying alcohol of bacteriochlorophyll *b* from *Ectothiorhodospira halochloris*, Z. Naturforsch. 36c (1981) 417–420.
[14] M. Kobayashi, H. Oh-oka, S. Akutsu, M. Akiyama, K. Tominaga, H. Kise, F. Nishida, T. Watanabe, The primary electron acceptor of green sulfur bacteria, bacteriochlorophyll 663, is chlorophyll *a* esterified with Δ2,6-phytadienol, Photosynth. Res. 63 (2000) 269–280.
[15] A.W. Roszak, T.D. Howard, J. Southall, A.T. Gardiner, C.J. Law, N.W. Isaacs, R.J. Cogdell, Crystal structure of the RC-LH1 core complex from *Rhodopseudomonas palustris*, Science 302 (2003) 1969–1972.
[16] M. van der Rest, G. Gingras, The pigment complement of the photosynthetic reaction center isolated from *Rhodospirillum rubrum*, J. Biol. Chem. 249 (1974) 6446–6453.
[17] G. Gingras, A comparative review of photochemical reaction center preparations from photosynthetic bacteria, in: R.K. Clayton, W.R. Sistrom (Eds.), The Photosynthetic Bacteria, Plenum Press, New York, 1978, pp. 119–131, Chap. 6.
[18] J.F. Imhoff, Taxonomy and physiology of phototrophic purple bacteria and green sulfur bacteria, in: R.E. Blankenship, M.T. Madigan, C.E. Bauer (Eds.), Anoxygenic Photosynthetic Bacteria, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1995, pp. 1–15.
[19] 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.
[20] T. Mizoguchi, M. Isaji, J. Harada, H. Tamiaki, Identification of 3,4-didehydrorhodopin as major carotenoid in *Rhodopseudomonas* species, Photochem. Photobiol. Sci. 7 (2008) 492–497.
[21] P. Qian, T. Yagura, Y. Koyama, R.J. Cogdell, Isolation and purification of the reaction center (RC) and the core (RC-LH1) complex from *Rhodobium marinum*: the LH1 ring of the detergent-solubilized core complex contains 32 bacteriochlorophylls, Plant Cell Physiol. 41 (2000) 1347–1353.
[22] K. Sauer, L.A. Austin, Bacteriochlorophyll-protein complexes from the light-harvesting antenna of photosynthetic bacteria, Biochemistry 17 (1978) 2011–2019.
[23] T. Mizoguchi, M. Isaji, J. Harada, K. Watabe, H. Tamiaki, Structural determination of the Δ2,10-phytadienyl substituent in the 17-propionate of bacteriochlorophyll-*b* from *Halorhodospira halochloris*, J. Porphyrins Phthalocyanines 13 (2009) 41–50.
[24] 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, Germany, 2008, pp. 275–293.
[25] 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.