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In Vitro Enzymatic Activities of Bacteriochlorophyll *a* Synthase Derived from the Green Sulfur Photosynthetic Bacterium *Chlorobaculum tepidum*

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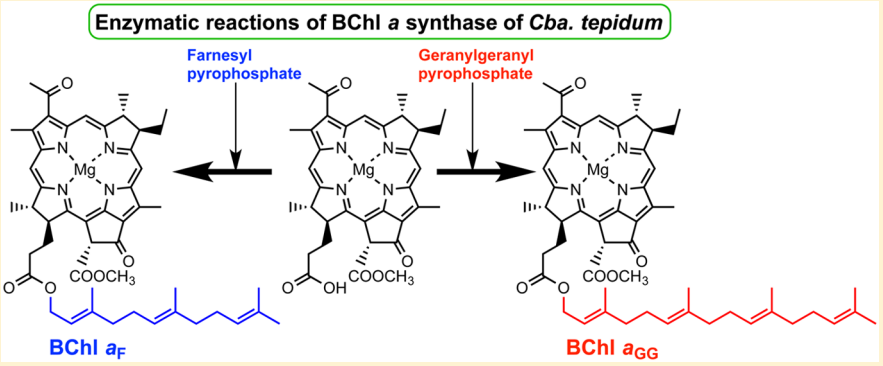

ABSTRACT: The activity of an enzyme encoded by the *CT1610* gene in the green sulfur photosynthetic bacterium *Chlorobaculum tepidum*, which was annotated as bacteriochlorophyll (BChl) *a* synthase, BchG (denoted as tepBchG), was examined *in vitro* using the lysates of *Escherichia coli* containing the heterologously expressed enzyme. BChl *a* possessing a geranylgeranyl group at the 17-propionate residue (BChl a_{GG}) was produced from bacteriochlorophyllide (BChlide) *a* and geranylgeranyl pyrophosphate in the presence of tepBchG. Surprisingly, tepBchG catalyzed the formation of BChl *a* bearing a farnesyl group (BChl a_F) as in the enzymatic production of BChl a_{GG}, indicating loose recognition of isoprenoid pyrophosphates in tepBchG. In contrast to such loose recognition of isoprenoid substrates, BChlide *c* and chlorophyllide *a* gave no esterifying product upon being incubated with geranylgeranyl or farnesyl pyrophosphate in the presence of tepBchG. These results confirm that tepBchG undoubtedly acts as the BChl *a* synthase in *Cba. tepidum*. The enzymatic activity of tepBchG was higher than that of BchG of *Rhodobacter sphaeroides* at 45 °C, although the former activity was lower than the latter below 35 °C.

Chlorophyll (Chl) and bacteriochlorophyll (BChl) pigments are crucial in photosynthetic light-harvesting and charge-separating processes. These pigments generally consist of a cyclic tetrapyrrole with an exocyclic five-membered E-ring, and, except for Chls *c*, possess a long esterifying hydrocarbon chain at the 17-propionate residue.[1,2] The esterifying groups are not conjugated with the photofunctional cyclic tetrapyrrole π-skeletons in chlorophyllous pigments; they play a major role in the formation and stability of photosynthetic apparatus by hydrophobic interactions with their surrounding microenvironments. The attachments of esterifying moieties to the free 17^2-carboxy forms of (B)Chls, named chlorophyllides (Chlides) and bacteriochlorophyllides (BChlides), are catalyzed by enzymes called (B)Chl synthases in the last step of the (B)Chl biosynthetic pathways.[3−7]

The esterifying chains of chlorophyllous pigments are full of variety in green sulfur photosynthetic bacteria, which possess three kinds of such pigments, namely, major light-harvesting BChl species (BChl *c*, *d*, and *e*) esterified with farnesol (BChl c_F, d_F, and e_F), BChl *a* esterified with phytol (BChl a_P), and Chl *a* esterified with Δ2,6-phytadienol (Chl a_{PD}).[8−12] The molecular structures of (B)Chls found in one of the green sulfur bacteria, *Chlorobaculum tepidum*, are shown in Figure 1. BChl c_F, d_F, or e_F is most abundant [~97% of total (B)Chls] in cells of green sulfur bacteria and forms self-aggregates in extramembranous antenna complexes, called chlorosomes.[8−14] In contrast, the amounts of BChl a_P and Chl a_{PD} in green sulfur bacteria are small [~3 and ~0.3% of total (B)Chls, respectively].[9] BChl a_P is present in both reaction center and antenna complexes, whereas Chl a_{PD} functions as the primary electron acceptor in the reaction center.[8,9,11,12,15] Such a variety of esterifying groups of (B)Chl pigments in green sulfur bacteria is in sharp contrast to the homogeneity of the 17^4-

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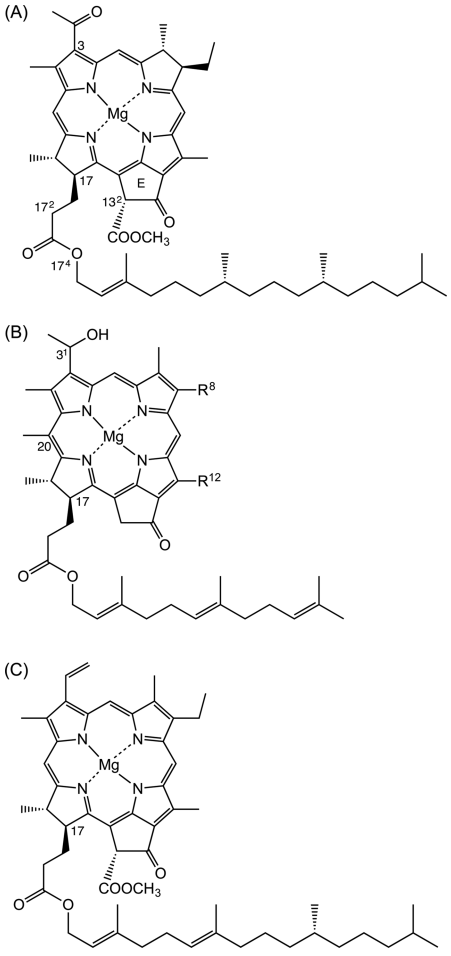

Figure 1. Molecular structures of three chlorophyllous pigments in *Cba. tepidum*: (A) BChl a_P, (B) BChl c_F, where $R^8 = C_2H_5$, *n*-C_3H_7, or iso-C_4H_9 and $R^{12} = CH_3$ or C_2H_5, and (C) Chl a_{PD}.

substituents of chlorophyllous pigments in many photosynthetic organisms.[1,2]

Three types of (B)Chl synthases, BchK, BchG, and ChlG, were annotated by genome analysis of *Cba. tepidum* and are thought to participate in the attachments of long hydrocarbon chains to the 17^2-carboxy group in three (B)Chlides.[9,11,12,16−18] Insertional inactivation of the *bchK* gene in *Cba. tepidum* produced no BChl *c*, indicating that BchK catalyzes the connection of a farnesyl group with BChlide *c*.[16] The BchG encoded by the *CT1610* gene found in the genomic DNA of *Cba. tepidum* was putatively assigned to BChl *a* synthase on the basis of the results of complementation of a Δ*bchG* mutant of *Rhodobacter capsulatus* with the *CT1610* gene.[19] However, there is no information about the enzymatic activities of BchG of *Cba. tepidum*. *In vitro* assays of enzymes working in the pigment biosynthetic pathway of photosynthetic organisms provide direct evidence of their functions and allow us to understand their substrate specificity and enzymatic activities clearly.[20−36] Actually, *in vitro* enzymatic activities of (B)Chl synthases of higher plants,[28−32] cyanobacteria,[33,34] purple photosynthetic bacteria,[33−35] and a filamentous anoxygenic phototroph[36] have been reported and provided useful information about their functions. In this study, we first report enzymatic assays of BchG of *Cba. tepidum*, which was heterologously expressed in *Escherichia coli*, and compare its activities with those of BChl *a* synthase from *Rba. sphaeroides*.

MATERIALS AND METHODS

Apparatus. High-performance liquid chromatography (HPLC) analysis was performed with a Shimadzu LC-20AT pump and an SPD-M20A detector. Liquid chromatography−mass spectrometry (LC−MS) was conducted with a Shimadzu LCMS-2020 system equipped with an electrospray ionization probe. Mass spectrometry was conducted with a Shimadzu AXIMA confidence mass spectrometer. Visible absorption spectra were measured with a Shimadzu UV-2450 spectrophotometer.

Materials. BChlide *a* and Chlide *a* were prepared from BChl *a* and Chl *a*, which were extracted from a purple photosynthetic bacterium *Rba. sphaeroides* and a cyanobacterium *Spirulina geitleri*, respectively, by use of a lysate of *E. coli* that expressed chlorophyllase.[26,37,38] A mixture of BChlide *c* homologues (various 8- and 12-alkylation) and epimers (3^1R and 3^1S stereochemistry) was prepared by an alkaline hydrolysis of the esterifying moiety of a BChl *c* homologue mixture, which was isolated from *Cba. tepidum*, as follows. A BChl *c* homologue mixture was incubated in a 4/1 (v/v) acetone/water mixture containing 0.2 M NaOH overnight at room temperature in the dark. After removal of unreacted BChl *c* with hexane, BChlide *c* was extracted with diethyl ether. The organic solution was washed with water, followed by evaporation. Geranylgeranyl pyrophosphate and farnesyl pyrophosphate in a methanol/aqueous NH_4OH mixture (1 mg mL^{-1}) were purchased from Sigma-Aldrich and used as supplied.

Plasmid Construction. BchG of *Cba. tepidum* and BchG of *Rba. sphaeroides* are hereafter denoted tepBchG and sphBchG, respectively. To express tepBchG and sphBchG in *E. coli*, expression plasmids pET21-tepBchG and pET21-sphBchG, respectively, were constructed as follows. For the construction of pET21-tepBchG, the polymerase chain reaction (PCR) was performed to amplify the *bchG* gene of *Cba. tepidum* using a primer set of tbchG-F (GAAGGAGATATACATATGAACGGAAGCGATACGCT) and tbchG-R (GCTCGAATTCGGATCTTACGGCCTTATGCCGACC), with KOD -plus- DNA polymerase (TOYOBO). The underlined sequences in an oligonucleotide primer represent overlapping regions of the cloning site of expression vector pET21(a)$^+$ (Novagen, Merck KGaA, Darmstadt, Germany). The pET21(a)$^+$ primer was digested with NdeI and BamHI and then mixed with the PCR product described above containing *bchG* of *Cba. tepidum*. Their fragments were ligated using an In-Fusion HD Cloning Kit (Clontech, Mountain View, CA), producing plasmid pET21-tepBchG. Plasmid pET21-sphBchG was constructed by the same method using the sbchG-F (GAAGGAGATATACAT/ATGAGTGTCAATCTATCCTTAC) and sbchG-R (GCTCGAATTCGGATC/TCACGGCAGCACCTCCAG) primers. DNA sequence analysis for confirmation of *bchG* genes in the constructed plasmids was performed using primers T7 promoter and T7 terminator (TaKaTa Bio Inc.).

Preparation of BchG. For the *in vitro* enzymatic assays of BChl *a* synthases, the cell lysates containing active tepBchG and sphBchG were obtained by expression in *E. coli* Bl21(DE3) strain. The 2.5 mL overnight cultures of *E. coli* Bl21(DE3) containing pET21-tepBchG or pET21-sphBchG were inoculated with 250 mL of TB medium containing ampicillin (final concentration of 100 μg mL^{-1}) and grown at 37 °C for 4 h. Isopropyl 1-thio-β-D-galactopyranoside was added to the culture (final concentration of 0.5 mM) for induction of

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bchG gene expression, followed by overnight incubation at 28 °C. The harvested cells were suspended in a buffer containing 50 mM Tris-HCl (pH 7.8) and 150 mM NaCl and disrupted with sonication. After centrifugation at 10322*g* for 15 min at 4 °C, the supernatants were used for enzymatic assays.

Enzymatic Assays. Enzymatic assays of BchG proteins were performed as described in the previous report.[33] A 50 mM HEPES-NaOH buffer (pH 6.5−8.5) containing 120 mM potassium acetate and 10 mM magnesium acetate (1 mL) was mixed with glycerol (100 μL) and 2-mercaptoethanol (1 μL), followed by addition of isoprenoid pyrophosphate (geranylgeranyl or farnesyl pyrophosphate, typically 10 μL), an acetone solution of (B)Chlide (0.1 mM, 50 μL), and the lysate of *E. coli* containing BchG (amount of protein in the reaction solution, 1.5 mg). After the reaction mixture had been incubated at an appropriate temperature controlled by a water bath in the dark, chlorophyllous pigments possessing an isoprenoid moiety were extracted with diethyl ether and dried with nitrogen gas. The pigments were analyzed on a $5C_{18}$-AR-II HPLC column (6 mmϕ × 250 mm) with methanol at a flow rate of 1.0 mL min^{-1}. The relative yields from BChlide *a* to BChl *a* were represented against the yield of BChl a_{GG} using tepBchG under the reaction conditions at pH 7.5 and 35 °C for 30 min.

RESULTS

Substrate Specificity. Figure 2A shows a typical elution pattern of the extracts from the reaction solution containing BChlide *a* and geranylgeranyl pyrophosphate in the presence of tepBchG at pH 7.5 and 35 °C. A main product was eluted at 9.5 min in this chromatogram. This product had Soret, Q_x, and Q_y absorption bands at 364, 608, and 770 nm, respectively, in the HPLC eluent, indicating that the bacteriochlorin macrocycle was intact in this product. This product gave a molecular ion peak at *m*/*z* 905.4 in its LC−MS analysis, whose value was almost identical to the calculated value of BChl a_{GG} (*m*/*z* 905.50 for MH^+). In the control experiment using the lysate of *E. coli* containing the empty pET21(a)$^+$ vector, no fraction derived from BChl a_{GG} was detected (Figure 2C). These data show that BChl a_{GG} is formed by the catalytic action of tepBchG. The fraction at 4.8 min was ascribed to unreacted BChlide *a*, and the slight fractions eluted around 5−6 min were denatured compounds of BChlide *a*, including its demetalated (pheophytinization) species, bacteriopheophorbide (BPheide) *a*. A small amount of allomerized BChl a_{GG} (a 13^2-hydroxy substitute of BChl a_{GG}), judged from its online absorption and MS spectra, was detected at 8.2 min.

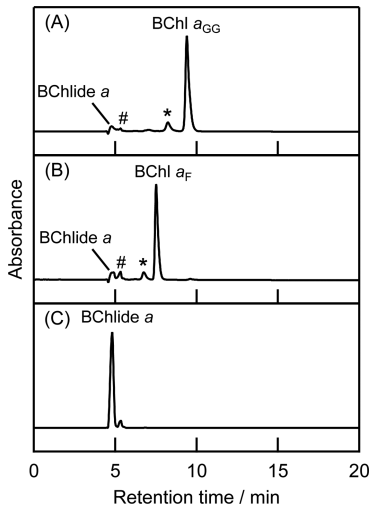

Figure 2. HPLC elution patterns of the extracts from reaction solutions of BChlide *a* and geranylgeranyl pyrophosphate (A) and those of BChlide *a* and farnesyl pyrophosphate (B) incubated with tepBchG, and those of BChlide *a* and geranylgeranyl pyrophosphate incubated with the lysate of *E. coli* containing the empty pET21(a)$^+$ vector (C) at pH 7.5 and 35 °C for 30 min. The chromatograms were recorded at 770 nm and normalized at the largest peaks. The fractions denoted by number signs and asterisks were ascribed to denatured compounds of BChlide *a* and allomerized BChl *a*, respectively.

Farnesyl (C15) pyrophosphate was incubated instead of geranylgeranyl (C20) pyrophosphate with BChlide *a* in the presence of tepBchG. Figure 2B depicts a typical HPLC elution pattern of the products extracted from the reaction solution containing BChlide *a* and farnesyl pyrophosphate. A major product, whose Soret, Q_x, and Q_y bands were positioned at 364, 608, and 770 nm, respectively, was detected at 7.5 min. This product eluted earlier than BChl a_{GG}. LC−MS analysis demonstrated that this product exhibited a molecular ion peak at *m*/*z* 837.6, which corresponded to the calculated value of BChl *a* possessing a farnesyl group (BChl a_F, *m*/*z* 837.44 for MH^+). These results allow us to assign this main product to BChl a_F. Extracted BChlide *a* and its denatured pigments as well as slightly allomerized BChl a_F were also observed around 5−6 min and at 6.8 min as seen in the case of the enzymatic reaction using geranylgeranyl pyrophosphate.

Time courses of the relative yields of BChl a_{GG} and BChl a_F by tepBchG were compared in Figure 3. The relative yields were represented against the yield of BChl a_{GG} using tepBchG for 30 min, as described above. The formation of BChl a_F was faster than that of BChl a_{GG} in the early phase, and the relative yields became similar to each other after 15 min in their time courses. The relative yield of BChl a_F to that of BChl a_{GG} by tepBchG was estimated to be 0.92 after incubation for 30 min. These values indicate that tepBchG can attach a shorter farnesyl moiety to the 17^2-carboxy group of BChlide *a* at the similar level as in the case of a geranylgeranyl moiety. Therefore, tepBchG can latently utilize farnesyl pyrophosphate as a substrate in addition to geranylgeranyl pyrophosphate, although BChl a_F is not biosynthesized *in vivo*.

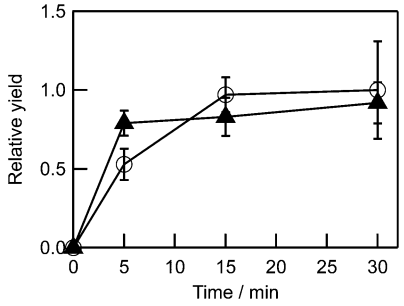

Figure 3. Time courses of the formation of BChl a_{GG} (○) and BChl a_F (▲) by tepBchG at pH 7.5 and 35 °C. The yields were the averaged values of three to six independent measurements and were normalized at the value of BChl a_{GG} by incubation for 30 min.

Figure 4 shows the dependence of the concentrations of two isoprenoid pyrophosphates on the enzymatic activities of tepBchG after incubation at pH 7.5 and 35 °C for 5 min. The relative yields of BChl a_F from BChlide *a* and farnesyl

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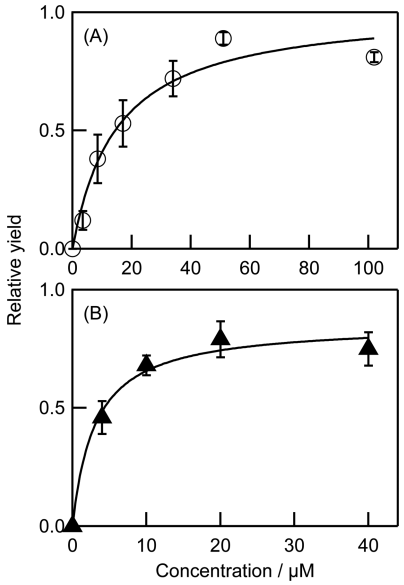

Figure 4. Dependence of concentrations of geranylgeranyl (A) and farnesyl pyrophosphates (B) on the conversion from BChlide *a* to BChl a_{GG} and BChl a_F, respectively, by tepBchG after incubation at pH 7.5 and 35 °C for 5 min. The yields were the averaged values of three or four independent measurements and were normalized at the value of BChl a_{GG} after incubation at pH 7.5 and 35 °C for 30 min.

pyrophosphate from tepBchG were higher than those of BChl a_{GG} by using geranylgeranyl pyrophosphate when concentrations of the isoprenoid diphosphates were low. In a while, the enzymatic activities of tepBchG by using geranylgeranyl pyrophosphate as a substrate reached levels slightly higher than those with farnesyl pyrophosphate. An apparent Michaelis constant K_m of tepBchG with geranylgeranyl pyrophosphate was 5 times larger than that with farnesyl pyrophosphate.

Activities of sphBchG were also examined using BChlide *a* and the two types of isoprenoid pyrophosphates mentioned above. Panels A and B of Figure 5 show typical HPLC elution patterns of the products after incubation of geranylgeranyl and farnesyl pyrophosphates with BChlide *a* in the presence of sphBchG, respectively. BChl a_{GG} was eluted at 9.5 min (Figure 5A), whereas BChl a_F was detected at 7.5 min (Figure 5B). In

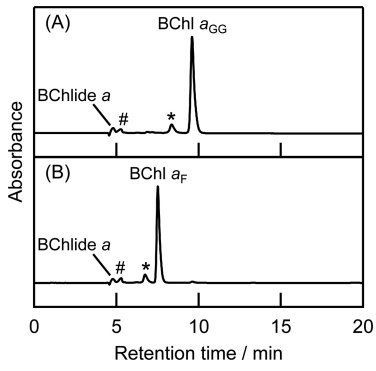

Figure 5. HPLC elution patterns of the extracts from reaction solutions of BChlide *a* and geranylgeranyl pyrophosphate (A) and those of BChlide *a* and farnesyl pyrophosphate (B) incubated with sphBchG at pH 7.5 and 35 °C for 30 min. The chromatograms were recorded at 770 nm and normalized at the largest peaks. The fractions denoted by number signs and asterisks were ascribed to denatured compounds of BChlide *a* and allomerized BChl *a*, respectively.

both chromatograms, BChlide *a*, its denatured pigments, and allomerized BChl *a* were observed at levels similar to those in the assays of tepBchG. The relative yield of BChl a_F to that of BChl a_{GG} by sphBchG was estimated to be 1.0, which was similar to that by tepBchG. Therefore, sphBchG can also utilize farnesyl pyrophosphate as well as geranylgeranyl pyrophosphate.

BChlide *c*, which is a biosynthetic precursor (17^2-carboxy form) of BChl c_F *in vivo*, was incubated with two isoprenoid pyrophosphates in the presence of tepBchG. Panels A and B of Figure 6 show typical HPLC chromatograms of the extracts

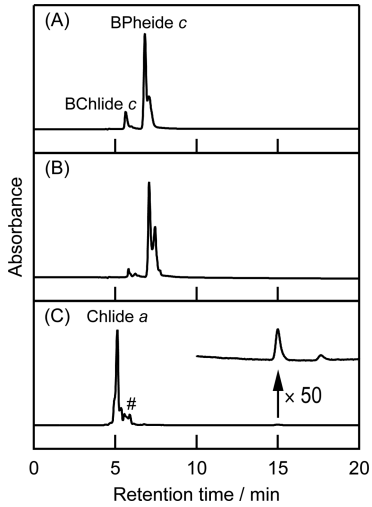

Figure 6. HPLC elution patterns of the extracts from reaction solutions of BChlide *c* and geranylgeranyl pyrophosphate (A), those of BChlide *c* and farnesyl pyrophosphate (B), and those of Chlide *a* and geranylgeranyl pyrophosphate (C) incubated with tepBchG at pH 7.5 and 35 °C for 30 min. Chromatograms A–C were recorded at 668, 668, and 665 nm, respectively, and normalized at the largest peaks. The fraction denoted by a number sign in panel C was ascribed to denatured compounds of Chlide *a*. A magnified chromatogram of the extracts of Chlide *a* and geranylgeranyl pyrophosphate from 10 to 20 min is inset in panel C.

from the reaction solutions containing BChlide *c* with geranylgeranyl and farnesyl pyrophosphates, respectively. The fractions that eluted around 6 and 7 min were assigned to a mixture of BChlide *c* homologue/epimers and the corresponding BPheide *c* mixture, respectively. Demetalation of BChlide *c* through the reaction occurred to a greater extent than that of BChlide *a* and Chlide *a* under the conditions presented here. This is in line with inherent demetalation properties of BChl *c*; BChl *c* is demetalated more rapidly than Chl *a* and BChl *a* because of the presence of the methyl group at position 20 of the chlorin macrocycle.[39−42] BPheide *c* was extracted more effectively than BChlide *c* with diethyl ether, resulting in its significant detection in these chromatograms, in which the amount of BPheide *c* was ∼10 times larger than that of BChlide *c*. Further degradation of BChlide *c* tended to occur at higher temperatures,[40] which cannot allow us to examine the assay using BChlide *c* at 45 °C. No BChl c_{GG} and BChl c_F were detected around 10−15 min in these chromatograms, indicating that tepBchG cannot catalyze the attachments of isoprenoid chains to BChlide *c*. Figure 6C shows a typical elution pattern of the extracts from the reaction solution for the enzymatic assay using Chlide *a* and geranylgeranyl pyrophosphate. The fraction at 5.1 min could be ascribed to Chlide *a*,

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which was extracted from the reaction solution. Degraded pigments of Chlide *a*, including demetalated pheophorbide *a*, appeared after the fraction of Chlide *a* around 6 min. In the case of Chlide *a* as a substrate, Chl a_{GG} was hardly observed around 15 min at both reaction temperatures, 35 and 45 °C; the amount of a pigment that eluted at 15 min (inset in Figure 6C) was too small to characterize under our analytical conditions. These results clearly prove that tepBchG is the BChl *a* synthase in *Cba. tepidum* and can hardly use the other two chlorophyllous pigments, which exist in this bacterium.

Comparison of Enzymatic Activities between tepBchG and sphBchG. The effects of reaction temperature and pH on the production of BChl a_{GG} by tepBchG and sphBchG were examined by enzymatic reactions for 30 min. The plot with empty circles in Figure 7 depicts temperature-dependent conversion efficiencies from BChlide *a* to BChl a_{GG} by tepBchG between 25 and 55 °C. The relative yields increased with reaction temperature up to 45 °C and significantly decreased at 55 °C. The temperature at which tepBchG exhibited the highest activities was very close to the optimal growth temperature of *Cba. tepidum*.[43] The pH dependency of the relative yields between pH 6.5 and 8.5 is represented as the plot with empty circles in Figure 8. The activity of the enzyme tepBchG exhibited a slight pH dependence; namely, the relative yield was slightly smaller at pH 6.5 than at pH 7.5 and 8.5.

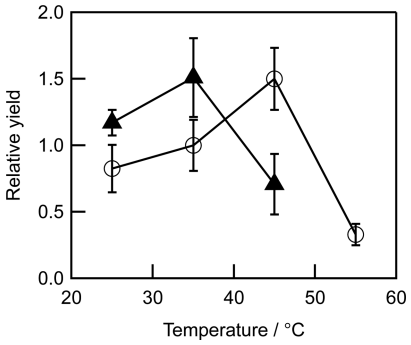

Figure 7. Temperature-dependent efficiencies of the conversion from BChlide *a* to BChl a_{GG} by tepBchG (○) and sphBchG (▲) by incubation at pH 7.5 for 30 min. The yields were the averaged values of three to seven independent measurements and were normalized to the values of tepBchG at 35 °C.

The plots with filled triangles in Figures 7 and 8 represent the temperature and pH dependence of the relative yields of BChl a_{GG} by catalytic actions of sphBchG. The sphBchG enzyme exhibited the highest efficiency at 35 °C. The ratios of the efficiency by tepBchG at pH 7.5 to that by sphBchG are estimated to be 0.71, 0.66, and 2.1 at 25, 35, and 45 °C, respectively. Therefore, tepBchG has enzymatic activity that is lower than that of sphBchG at 25 and 35 °C, but approximately 2 times higher activity at 45 °C. The difference in the temperature dependency of enzymatic activities between tepBchG and sphBchG suggests that both enzymes were adapted to the optimized growth temperature of each host organism. The relative yield by sphBchG at pH 7.5 was slightly larger than those at pH 6.5 and 8.5.

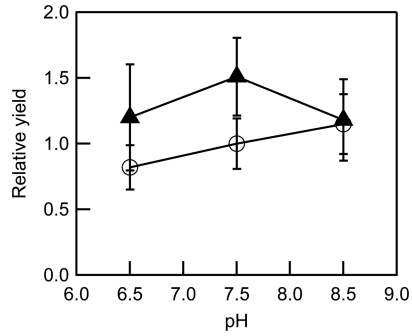

Figure 8. pH-dependent efficiencies of the conversion from BChlide *a* to BChl a_{GG} by tepBchG (○) and sphBchG (▲) by incubation at 35 °C for 30 min. The yields were the averaged values of four to seven independent measurements and were normalized to the values of tepBchG at pH 7.5.

DISCUSSION

This study first demonstrates *in vitro* enzymatic activities of BchG of the green sulfur bacterium *Cba. tepidum*, which possesses three kinds of chlorophyllous pigments esterified with different alcohols, namely, BChl c_F, BChl a_P, and Chl a_{PD}. The enzyme tepBchG catalyzed conversion of BChlide *a* to BChl a_{GG} but was unable to utilize BChlide *c* and Chlide *a* as substrates. These results clearly indicate that tepBchG functions as BChl *a* synthase in *Cba. tepidum*. This confirmation is consistent with no report of the inactivation of the *CT1610* gene in *Cba. tepidum*, because such inactivation would become lethal because of the essential roles played by BChl a_P in the reaction centers and possibly light-harvesting complex. These results are in line with the previous reports that BchGs of purple photosynthetic bacteria and a filamentous anoxygenic phototroph are specific to BChlide *a* and cannot utilize Chlide *a*.[33,34,36] It was suggested that BchG of a filamentous anoxygenic phototroph *Chloroflexus aurantiacus* recognized the 3-acetyl group of BChlide *a* without discriminating the skeleton of the tetrapyrrole macrocycles.[36] The enzyme tepBchG would differentiate BChlide *a* from BChlide *c* and Chlide *a* in the similar manner, in which the substituent at position 3 in the cyclic tetrapyrroles is one of the recognition moieties by BchG.

The enzymatic assays presented here indicate that both tepBchG and sphBchG can attach not only a geranylgeranyl moiety but also a farnesyl moiety to the 17-propionate residue of BChlide *a*. These suggest that BChl *a* synthases barely recognize the length of hydrocarbon chains, although (B)-Chlides are strictly recognized in these enzymes. Such loose recognition of isoprenoid moieties is in line with promiscuous isoprenoid recognition of an intramembranous prenyltransferase UbiA,[44−46] which belongs to the superfamily that includes Chl synthases. This prenyltransferase catalyzes the attachment of isoprenoid moieties to *p*-hydroxybenzoate and hardly recognizes isoprenoid moieties with various chain lengths. The recent three-dimensional crystal structure of UbiA proved the promiscuous recognition of isoprenoid moieties with this enzyme.[47] The sequence homologies of tepBchG and sphBchG were 35.1 and 34.0%, respectively, with UbiA from *Aeropyrum pernix*. Three amino acid residues (Arg67, Tyr115, and Asp186 in UbiA) of the seven important residues that participate in recognition of the pyrophosphate moiety in isoprenoid substrate are conserved in tepBchG and sphBchG. These suggest that tepBchG and sphBchG have manners of binding of isoprenoid pyrophosphate similar to those of UbiA, in which an isoprenoid chain sticks out of the cavity of BChl *a* synthases, although there is no structural information about (B)Chl

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synthases in photosynthetic organisms. It is worth noting that *Cba. tepidum* can biosynthesize BChl *c* derivatives esterified with various long-chain alcohols,[48−52] suggesting that BChl *c* synthase also promiscuously recognizes hydrocarbon moieties as in the case of BChl *a* synthase.

We should pay attention to discuss kinetically the enzymatic activities of tepBchG in these assays. First, these assays were performed in heterogeneous systems using the lysates of *E. coli* containing the heterologously expressed enzymes. Second, isoprenoid substrates might be partially aggregated because of their amphiphilic properties. It is noted that the predicted molar solubility of geranylgeranyl pyrophosphate (0.87−2.22 M at pH 6−8, data from SciFinder) was somewhat different from that of farnesyl pyrophosphate (2.62 M at pH 6−8, data from SciFinder). Therefore, the states of the two isoprenoid diphosphates in reaction buffers might affect the kinetic properties of the formation of BChls a_{GG} and a_F by tepBchG.

The similarity of *in vitro* catalytic activities of tepBchG using geranylgeranyl and farnesyl pyrophosphates would contradict the failure to detect BChl a_F in the cells of *Cba. tepidum*; the geranylgeranyl group of produced BChl a_{GG} is regio- and stereoselectively hydrogenated three times to give BChl a_P *in vivo*. It is too early to propose the mechanisms for the *in vivo* differentiability between geranylgeranyl and farnesyl pyrophosphates in the attachment of BChlide *a*, but we wish to make hypotheses about this enigma as follows. One is compartmentalization of the sites for the attachment of a geranylgeranyl moiety during BChl *a* biosynthesis and that of a farnesyl moiety during BChl *c* biosynthesis in *Cba. tepidum*. BChl a_{GG} would be selectively biosynthesized, if geranylgeranyl pyrophosphate is produced around tepBchG and farnesyl pyrophosphate is almost absent in the domain of BChl *a* biosynthesis. In a while, BChl c_F would be biosynthesized and aggregated in chlorosomes, if BChl *c* synthase, BchK, might be located and be able to utilize farnesyl pyrophosphate close to the chlorosomal membranes. It is possible that the concentration of farnesyl pyrophosphate would be below the level required for the attachment of this isoprenoid substrate to BChlide *a* at a significant rate in *Cba. tepidum* cells. Farnesyl pyrophosphate is majorly utilized in BChl *c* biosynthesis by BchK. Thus, it is possible that BchK has a higher affinity for farnesyl pyrophosphate and faster velocity of BChl *c* synthesis, as well, resulting in keeping the concentration of farnesyl pyrophosphate low in cells. One other is the potential instability of photosynthetic proteins that possess BChl a_F *in vivo*. It was reported that BChl a_{GG} accumulated in *Cba. tepidum* instead of BChl a_P by insertional inactivation of the *bchP* gene;[53,54] however, the amount of FMO proteins (situated between chlorosomes and reaction center complexes in *Cba. tepidum*) in the *bchP*-deleted cells decreased, and the variant FMO protein containing BChl a_{GG} was thermally less stable than the wild-type protein with BChl a_P.[55] The substitution of BChl a_P with BChl a_{GG} was also reported to decrease the amount of LH2 proteins carrying BChl *a* in purple photosynthetic bacterium *Rba. sphaeroides*, probably because of the instability of LH2 that binds BChl a_{GG} instead of BChl a_P.[2,56,57] These imply that BChl a_F−protein complexes are degraded *in vivo* because of their instability induced by the substitution of the esterifying group in BChl *a*. It should be noted that the possibility of discrimination of BChls *a* esterified with different alcohols by photosynthetic apparatus cannot be ruled out. Other reasons such as the participation of subsidiary pigment-binding carrier proteins[58−61] might be taken into consideration to tackle this enigma. The discrepancy between the *in vitro* loose specificity of isoprenoid moieties of BChl *a* synthases and no accumulation of BChl a_F *in vivo* will be a clue in understanding biosynthesis of chlorophyllous pigments and regulation of their amounts and localization in green photosynthetic bacteria.

CONCLUSION

This study clearly verifies that tepBchG is the enzymatic catalyst of condensation of the isoprenoid moiety with the 17^2-carboxy group of BChlide *a* in *Cba. tepidum* and cannot use BChlide *c* and Chlide *a* as substrates. In contrast to the specificity for (B)Chlide substrates, both tepBchG and sphBchG can attach not only a geranylgeranyl chain but also a shorter farnesyl chain to BChlide *a*. The enzymatic activity of tepBchG will be useful for the elucidation of mechanisms of biosynthesis of (B)Chls and regulation of their amounts in *Cba. tepidum*.

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Notes

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ABBREVIATIONS

BChl, bacteriochlorophyll; BChl a_F, bacteriochlorophyll *a* esterified with farnesol; BChl a_{GG}, bacteriochlorophyll *a* esterified with geranylgeraniol; BChl a_P, bacteriochlorophyll *a* esterified with phytol; BChl c_F, bacteriochlorophyll *c* esterified with farnesol; BChlide, bacteriochlorophyllide; BPheide, bacteriopheophorbide; Chl, chlorophyll; Chl a_{PD}, chlorophyll *a* esterified with Δ2,6-phytadienol; Chlide, chlorophyllide; HPLC, high-performance liquid chromatography; LC−MS, liquid chromatography−mass spectrometry; sphBchG, BchG of *Rba. sphaeroides*; tepBchG, BchG of *Cba. tepidum*.

REFERENCES

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

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

(3) Beale, S. I. (1999) Enzymes of chlorophyll biosynthesis. *Photosynth. Res. 60*, 43−73.

(4) Rüdiger, W. (2003) The last step of chlorophyll synthesis. In *The Porphyrin Handbook* (Kadish, K. M., Smith, K. M., and Guilard, R., Eds.) Vol. *13*, pp 71−108, Academic Press, Amsterdam.

(5) Bollivar, D. W. (2007) Recent advances in chlorophyll biosynthesis. *Photosynth. Res. 90*, 173−194.

DOI: 10.1021/acs.biochem.5b00311
Biochemistry 2015, 54, 4998−5005

(6) Chew, A. G. M., and Bryant, D. A. (2007) Chlorophyll biosynthesis in bacteria: the origins of structural and functional diversity. *Annu. Rev. Microbiol. 61*, 113−129.
(7) Tanaka, A., and Tanaka, R. (2006) Chlorophyll metabolism. *Curr. Opin. Plant Biol. 9*, 248−255.
(8) Olson, J. M. (1998) Chlorophyll organization and function in green photosynthetic bacteria. *Photochem. Photobiol. 67*, 61−75.
(9) Frigaard, N.-U., Chew, A. G. M., Li, H., Maresca, J. A., and Bryant, D. A. (2003) *Chlorobium tepidum*: insight into the structure, physiology, and metabolism of a green sulfur bacterium derived from the complete genome sequence. *Photosynth. Res. 78*, 93−117.
(10) Saga, Y., Shibata, Y., and Tamiaki, H. (2010) Spectral properties of single light-harvesting complexes in bacterial photosynthesis. *J. Photochem. Photobiol., C 11*, 15−24.
(11) Bryant, D. A., Liu, Z., Li, T., Zhao, F., Garcia Costas, A. M., Klatt, C. G., Ward, D. M., Frigaard, N.-U., and Overmann, J. (2012) Comparative and functional genomics of anoxygenic green bacteria from the Taxa *Chlorobi*, *Chloroflexi*, and *Acidobacteria*. In *Functional Genomics and Evolution of Photosynthetic Systems* (Burnap, R. L., and Vermaas, W. F. J., Eds.) pp 47−102. Springer, Dordrecht, The Netherlands.
(12) Orf, G. S., and Blankenship, R. E. (2013) Chlorosome antenna complexes from green photosynthetic bacteria. *Photosynth. Res. 116*, 315−331.
(13) Montaño, G. A., Bowen, B. P., LaBelle, J. T., Woodbury, N. W., Pizziconi, V. B., and Blankenship, R. E. (2003) Characterization of *Chlorobium tepidum* chlorosomes: a calculation of bacteriochlorophyll *c* per chlorosomes and oligomer modeling. *Biophys. J. 85*, 2560−2565.
(14) Saga, Y., Shibata, Y., Itoh, S., and Tamiaki, H. (2007) Direct counting of submicrometer-sized photosynthetic apparatus dispersed in medium at cryogenic temperature by confocal laser fluorescence microscopy: estimation of the number of bacteriochlorophyll *c* in single light-harvesting antenna complexes chlorosomes of green photosynthetic bacteria. *J. Phys. Chem. B 111*, 12605−12609.
(15) Kobayashi, M., Akiyama, M., Kise, H., and Watanabe, T. (2006) Unusual tetrapyrrole pigments of photosynthetic antennae and reaction centers: Specially-tailored chlorophylls. In *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications* (Grimm, B., Porra, R. J., Rüdiger, W., and Scheer, H., Eds.) pp 55− 66. Springer, Dordrecht, The Netherlands.
(16) Frigaard, N.-U., Voigt, G. D., and Bryant, D. A. (2002) *Chlorobium tepidum* mutant lacking bacteriochlorophyll *c* made by inactivation of the *bchK* gene, encoding bacteriochlorophyll *c* synthase. *J. Bacteriol. 184*, 3368−3376.
(17) Eisen, J. A., Nelson, K. E., Paulsen, I. T., Heidelberg, J. F., Wu, M., Dodson, R. J., Deboy, R., Gwinn, M. L., Nelson, W. C., Haft, D. H., Hickey, E. K., Peterson, J. D., Durkin, A. S., Kolonay, J. L., Yang, F., Holt, I., Umayam, L. A., Mason, T., Brenner, M., Shea, T. P., Parksey, D., Nierman, W. C., Feldblyum, T. V., Hansen, C. L., Craven, M. B., Radune, D., Vamathevan, J., Khouri, H., White, O., Gruber, T. M., Ketchum, K. A., Venter, J. C., Tettelin, H., Bryant, D. A., and Fraser, C. (2002) The complete genome sequence of *Chlorobium tepidum* TLS, a photosynthetic, anaerobic, green-sulfur bacterium. *Proc. Natl. Acad. Sci. U. S. A. 99*, 9509−9514.
(18) Garcia-Gil, L. J., Gich, F. B., and Fuentes-Garcia, X. (2003) A comparative study of bchG from green photosynthetic bacteria. *Arch. Microbiol. 179*, 108−115.
(19) Xiong, J., Fischer, W. M., Inoue, K., Nakahara, M., and Bauer, C. E. (2000) Molecular evidence for the early evolution of photosynthesis. *Science 289*, 1724−1730.
(20) Holtorf, H., Reinbothe, S., Reinbothe, C., Bereza, B., and Apel, K. (1995) Two routes of chlorophyllide synthesis that are differently regulated by light in barley (*Hordeum vulgare* L.). *Proc. Natl. Acad. Sci. U. S. A. 92*, 3254−3258.
(21) Fujita, Y., and Bauer, C. E. (2000) Reconstitution of light-independent protochlorophyllide reductase from purified Bchl and BchN-BchB subunits − *in vitro* confirmation of nitrogenase-like features of a bacteriochlorophyll biosynthesis enzyme. *J. Biol. Chem. 275*, 23583−23588.
(22) Oster, U., Tanaka, R., Tanaka, A., and Rüdiger, W. (2000) Cloning and functional expression of the gene encoding the key enzyme for chlorophyll *b* biosynthesis (CAO) from *Arabidopsis thaliana*. *Plant J. 21*, 305−310.
(23) Harada, J., Saga, Y., Yaeda, Y., Oh-oka, H., and Tamiaki, H. (2005) *In vitro* activity of C-20 methyltransferase, BchU, involved in bacteriochlorophyll *c* biosynthetic pathway in green sulfur bacteria. *FEBS Lett. 579*, 1983−1987.
(24) Shimoda, Y., Ito, H., and Tanaka, A. (2012) Conversion of chlorophyll *b* to chlorophyll *a* precedes magnesium dechelation for protection against necrosis in Arabidopsis. *Plant J. 72*, 501−511.
(25) Tsukatani, Y., Yamamoto, H., Mizoguchi, T., Fujita, Y., and Tamiaki, H. (2013) Completion of biosynthetic pathways for bacteriochlorophyll *g* in *Heliobacterium modesticaldum*: the C8-ethylidene group formation. *Biochim. Biophys. Acta, Bioenerg. 1827*, 1200−1204.
(26) Tsukatani, Y., Yamamoto, H., Harada, J., Yoshitomi, T., Nomata, J., Kasahara, M., Mizoguchi, T., Fujita, Y., and Tamiaki, H. (2013) An unexpectedly branched biosynthetic pathway for bacteriochlorophyll *b* capable of absorbing near-infrared light. *Sci. Rep. 3*, 1217.
(27) Tsukatani, Y., Harada, J., Nomata, J., Yamamoto, H., Fujita, Y., Mizoguchi, T., and Tamiaki, H. (2015) *Rhodobacter sphaeroides* mutants overexpressing chlorophyllide *a* oxidoreductase of *Balsto-chloris viridis* elucidate functions of enzymes in late bacteriochlorophyll biosynthetic pathways. *Sci. Rep. 5*, 9741.
(28) Benz, J., and Rüdiger, W. (1981) Chlorophyll biosynthesis: Various chlorophyllides as exogenous substrates for chlorophyll synthetase. *Z. Naturforsch. 36c*, 51−57.
(29) Helfrich, M., and Rüdiger, W. (1992) Various metal-lopheophorbides as substrates for chlorophyll synthetase. *Z. Naturforsch. 47c*, 231−238.
(30) Helfrich, M., Schoch, S., Lempert, U., Cmiel, E., and Rüdiger, W. (1994) Chlorophyll synthetase cannot synthesize chlorophyll *a'*. *Eur. J. Biochem. 219*, 267−275.
(31) Oster, U., and Rüdiger, W. (1997) The G4 gene of *Arabidopsis thaliana* encodes a chlorophyll synthase of etiolated plants. *Bot. Acta 110*, 420−423.
(32) Rüdiger, W., Böhm, S., Helfrich, M., Schulz, S., and Schoch, S. (2005) Enzymes of the last steps of chlorophyll biosynthesis: modification of the substrate structure helps to understand the topology of the active centers. *Biochemistry 44*, 10864−10872.
(33) Oster, U., Bauer, C. E., and Rüdiger, W. (1997) Characterization of chlorophyll *a* and bacteriochlorophyll *a* synthases by heterologous expression in *Escherichia coli*. *J. Biol. Chem. 272*, 9671−9676.
(34) Kim, E.-J., and Lee, J. K. (2010) Competitive inhibitions of the chlorophyll synthase of *Synechocystis* sp. strain PCC 6803 by bacteriochlorophyllide *a* and the bacteriochlorophyll synthase of *Rhodobacter sphaeroides* by chlorophyllide *a*. *J. Bacteriol. 192*, 198−207.
(35) Addlesee, H. A., Fiedor, L., and Hunter, C. N. (2000) Physical mapping of bchG, orf427, and orf177 in the photosynthesis gene cluster of *Rhodobacter sphaeroides*: functional assignment of the bacteriochlorophyll synthetase gene. *J. Bacteriol. 182*, 3175−3182.
(36) Schoch, S., Oster, U., Mayer, K., Feick, R., and Rüdiger, W. (1999) Substrate specificity of overexpressed bacteriochlorophyll synthase from *Chloroflexus aurantiacus*. In *The Chloroplast: From Molecular Biology to Biotechnology* (Argyroudi-Akoyunoglou, J. H., and Senger, H., Eds.) pp 213−216. Kluwer Academic, Dordrecht, The Netherlands.
(37) Tsuchiya, T., Ohta, H., Okawa, K., Iwamatsu, A., Shimada, H., Masuda, T., and Takamiya, K. (1999) Cloning of chlorophyllase, the key enzyme in chlorophyll degradation: finding of a lipase motif and the induction by methyl jasmonate. *Proc. Natl. Acad. Sci. U. S. A. 96*, 15362−15367.
(38) Sadaoka, K., Shoji, S., Hirota, K., Tsukatani, Y., Yoshitomi, T., Tamiaki, H., Kashimura, S., and Saga, Y. (2013) Pheophytinization kinetics of chlorophyll *c* under weakly acidic conditions: effects of acrylic acid residue at the 17-position. *Bioorg. Med. Chem. 21*, 6915−6919.

DOI: 10.1021/acs.biochem.5b00311
Biochemistry 2015, 54, 4998−5005

(39) Saga, Y., Hirai, Y., and Tamiaki, H. (2007) Kinetic analysis of demetalation of bacteriochlorophyll *c* and *e* homologs purified from green sulfur photosynthetic bacteria. *FEBS Lett. 581*, 1847−1850.
(40) Hirai, Y., Tamiaki, H., Kashimura, S., and Saga, Y. (2009) Physicochemical studies of demetalation of light-harvesting bacteriochlorophyll isomers purified from green sulfur photosynthetic bacteria. *Photochem. Photobiol. 85*, 1140−1146.
(41) Hirai, Y., Tamiaki, H., Kashimura, S., and Saga, Y. (2009) Demetalation kinetics of natural chlorophylls purified from oxygenic photosynthetic organisms: effect of the formyl groups conjugated directly to the chlorin π-macrocycle. *Photochem. Photobiol. Sci. 8*, 1701−1709.
(42) Kobayashi, M., Yamamura, M., Akiyama, M., Kise, H., Inoue, K., Hara, M., Wakao, N., Yahara, K., and Watanabe, T. (1998) Acid resistance of Zn-bacteriochlorophyll *a* from an acidophilic bacterium *Acidiphilum rubrum*. *Anal. Sci. 14*, 1149−1152.
(43) Wahlund, T. M., Woese, C. R., Castenholz, R. W., and Madigan, M. T. (1991) A thermophilic green sulfur bacterium from New Zealand hot springs. *Arch. Microbiol. 156*, 81−90.
(44) Kalén, A., Appelkvist, E.-L., Chojnacki, T., and Dallner, G. (1990) Nonaprenyl-4-hydroxybenzoate transferase, an enzyme involved in ubiquinone biosynthesis, in the endoplasmic reticulum-golgi system of rat liver. *J. Biol. Chem. 265*, 1158−1164.
(45) Swiezewska, E., Dallner, G., Andersson, B., and Ernster, L. (1993) Biosynthesis of ubiquinone and plastoquinone in the endoplasmic reticulum-golgi membranes of spinach leaves. *J. Biol. Chem. 268*, 1494−1499.
(46) Suzuki, K., Ueda, M., Yuasa, M., Nakagawa, T., Kawamukai, M., and Matsuda, H. (1994) Evidence that *Escherichia coli ubiA* product is a functional homolog of yeast COQ2, and regulation of *ubiA* gene expression. *Biosci., Biotechnol., Biochem. 58*, 1814−1819.
(47) Cheng, W., and Li, W. (2014) Structural insights into ubiquinone biosynthesis in membranes. *Science 343*, 878−881.
(48) Caple, M. B., Chow, H., and Strouse, C. E. (1978) Photosynthetic pigments of green sulfur bacteria. The esterifying alcohols of bacteriochlorophylls *c* from *Chlorobium limicola*. *J. Biol. Chem. 253*, 6730−6737.
(49) Steensgaard, D. B., Cox, R. P., and Miller, M. (1996) Manipulation of the bacteriochlorophyll *c* homolog distribution in the green sulfur bacterium *Chlorobium tepidum*. *Photosynth. Res. 48*, 385−393.
(50) Mizoguchi, T., and Tamiaki, H. (2007) The effect of esterifying chains at the 17-propionate of bacteriochlorophylls-*c* on their self-assembly. *Bull. Chem. Soc. Jpn. 80*, 2196−2202.
(51) Nishimori, R., Mizoguchi, T., Tamiaki, H., Kashimura, S., and Saga, Y. (2011) Biosynthesis of unnatural bacteriochlorophyll *c* derivatives esterified with α,ω-diols in the green sulfur photosynthetic bacterium *Chlorobaculum tepidum*. *Biochemistry 50*, 7756−7764.
(52) Saga, Y., Hayashi, K., Mizoguchi, T., and Tamiaki, H. (2014) Biosynthesis of bacteriochlorophyll *c* derivatives possessing chlorine and bromine atoms at the terminus of esterifying chains in the green sulfur photosynthetic bacterium *Chlorobaculum tepidum*. *J. Biosci. Bioeng. 118*, 82−87.
(53) Harada, J., Miyago, S., Mizoguchi, T., Azai, C., Inoue, K., Tamiaki, H., and Oh-oka, H. (2008) Accumulation of chlorophyllous pigments esterified with the geranylgeranyl group and photosynthetic competence in the *CT2256*-deleted mutant of the green sulfur bacterium *Chlorobium tepidum*. *Photochem. Photobiol. Sci. 7*, 1179−1187.
(54) Gomez Maqueo Chew, A. G. M., Frigaard, N.-U., and Bryant, D. A. (2008) Identification of the *bchP* gene, encoding geranylgeranyl reductase in *Chlorobaculum tepidum*. *J. Bacteriol. 190*, 747−749.
(55) Wen, J., Harada, J., Buyle, K., Yuan, K., Tamiaki, H., Oh-oka, H., Loomis, R. A., and Blankenship, R. E. (2010) Characterization of an FMO variant of *Chlorobaculum tepidum* carrying bacteriochlorophyll *a* esterified by geranylgeraniol. *Biochemistry 49*, 5455−563.
(56) Addlesee, H. A., and Hunter, C. N. (1999) Physical mapping and functional assignment of the geranylgeranyl-bacteriochlorophyll reductase gene, *bchP*, of *Rhodobacter sphaeroides*. *J. Bacteriol. 181*, 7248−7255.
(57) Mizoguchi, T., Isaji, M., Harada, J., Tsukatani, Y., and Tamiaki, H. (2015) The 17-propionate esterifying variants of bacteriochlorophyll-*a* and bacteriopheophytin-*a* in purple photosynthetic bacteria. *J. Photochem. Photobiol., B 142*, 244−249.
(58) Larkin, R. M., Alonso, J. M., Ecker, J. R., and Chory, J. (2003) GUN4, a regulator of chlorophyll synthesis and intracellular signaling. *Science 299*, 902−906.
(59) Davison, P. A., Schubert, H. L., Reid, J. D., Iorg, C. D., Heroux, A., Hill, C. P., and Hunter, C. N. (2005) Structural and biochemical characterization of Gun4 suggests a mechanism for its role in chlorophyll biosynthesis. *Biochemistry 44*, 7603−7612.
(60) Chew, A. G. M., and Bryant, D. A. (2007) Characterization of a plant-like protochlorophyllide *a* divinyl reductase in green sulfur bacteria. *J. Biol. Chem. 282*, 2967−2975.
(61) Sawicki, A., and Willows, R. D. (2010) BchJ and BchM interact in a 1:1 ratio with the magnesium chelatase BchH subunit of *Rhodobacter capsulatus*. *FEBS J. 277*, 4709−4721.

DOI: 10.1021/acs.biochem.5b00311
Biochemistry 2015, 54, 4998−5005