Reconstitution of 3-Acetyl Chlorophyll a into Light-Harvesting Complex 2 from the Purple Photosynthetic Bacterium Phaeospirillum molischianum

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**ABSTRACT:** The manipulation of B800 bacteriochlorophyll (BChl) a in light-harvesting complex 2 (LH2) from the purple photosynthetic bacterium *Phaeospirillum molischianum* (molischianum-LH2) provides insight for understanding the energy transfer mechanism and the binding of cyclic tetrapyrroles in LH2 proteins since *molischianum*-LH2 is one of the two LH2 proteins whose atomic-resolution structures have been determined and is a representative of type-2 LH2 proteins. However, there is no report on the substitution of B800 BChl a in *molischianum*-LH2. We report the reconstitution of 3-acetyl chlorophyll (AcChl) a, which has a 17,18-dihydroporphyrin skeleton, to the B800 site in *molischianum*-LH2. The 3-acetyl group in AcChl a formed a hydrogen bond with β-Thr23 in essentially the same manner as native B800 BChl a, but this hydrogen bond was weaker than that of B800 BChl a. This change can be rationalized by invoking a small distortion in the orientation of the 3-acetyl group in the B800 cavity by dehydrogenation in the B-ring from BChl a. The energy transfer from AcChl a in the B800 site to B850 BChl a was about 5-fold slower than that from native B800 BChl a by a decrease of the spectral overlap between energy-donating AcChl a and energy-accepting B850 BChl a.

**INTRODUCTION**

Photosynthetic light-harvesting proteins are important in the primary process of conversion of light energy into chemical energy in nature. Chlorophyll (Chl) and bacteriochlorophyll (BChl) pigments play crucial roles in photon capture and excitation energy transfer (EET) in light-harvesting proteins.1−3 Chl pigments in oxygenic photosynthetic organisms generally have a 17,18-dihydroporphyrin skeleton. BChls a, b, and g, which are major pigments in purple photosynthetic bacteria and heliobacteria, consist of 7,8,17,18-tetrahydroporphyrin. The difference in the degree of hydrogenation in the cyclic tetapyrrole between Chls and BChls is primarily responsible for the photofunctions in light-harvesting proteins since the spectral coverage of (B)Chl pigments and their physicochemical features of the lowest energy absorption bands (Qy bands) depend on their cyclic tetapyrrole skeletons.4,5 Therefore, control of (de)hydrogenation of cyclic tetapyrrole pigments is a promising prospect for elucidating the photofunctional mechanisms of light-harvesting proteins and improving photocconversion efficiency in photosynthetic systems.

Light-harvesting complex 2 (LH2) is favorable for manipulating the cyclic tetapyrrole pigments of photosynthetic proteins in the investigation of the effects of (B)Chl structures with regard to their photosynthetic functions. LH2 proteins are peripheral antennas in purple photosynthetic bacteria. In these proteins, BChl a and carotenoids are circularly organized in the scaffold of transmembranous α- and β-polypeptides (Figure 1A).6−7 Two types of BChl a pigments, denoted as B800 and B850 based on their peak positions of Qy bands, are present in LH2 proteins. B800 BChl a is located between the β-polypeptides as the monomeric form. B850 BChl a pigments, which are sandwiched between the α- and β-polypeptides, are excitation-coupled with each other in their circular arrangement. Excitation energy is efficiently transferred from B800 to B850 BChl a in LH2 proteins.8−10 LH2 proteins from *Phaeospirillum* (Phs.) *molischianum* and *Rhodoblastus* (Rbl.) *acidiphilus*, denoted as *molischianum*-LH2 and *acidiphilus*-LH2, are the representative LH2 of purple photosynthetic bacteria since the three-dimensional structures of only these two LH2 proteins are available at atomic-level resolutions6−7 and LH2 proteins from various purple bacteria are classified based on their spectral properties into two types: type-1 LH2 (*acidiphilus*-LH2 like) and type-2 LH2 (*molischianum*-LH2 like).11 Therefore, *molischianum*-LH2 and...
Acidophillus-LH2 are key proteins to comprehensively understand the structural and functional principles of LH2 proteins. The binding pockets of B800 BChl a in LH2 have attracted considerable attention for demonstrating the importance of cyclic tetrapyrrole structures on (B)Chl binding and intra-molecular interactions. Therefore, the peripheral substituents in AcChl a are the same as BChl a; the structural difference between AcChl a and BChl a is restricted to the degree of hydrogenation in the B-ring of the cyclic tetrapyrroles. In spite of the small structural difference between AcChl a and BChl a, the Q band of AcChl a at 677 nm is largely shifted to a shorter wavelength than that of BChl a at 770 nm in acetone. AcChl a is thus suitable to change the photofunctions of LH2 proteins by its reconstitution into the B800 site in molischianum-LH2.

Figure 1. Overall structure (top view) (A) and the structure in the proximity of B800 BChl a in molischianum-LH2 (B). Protein Data Bank entry 1LGH. B800 and B850 BChl a are colored magenta and green, respectively. The α- and β-polypeptides are colored sky blue and orange, respectively. (C) Molecular structures of BChl a (single bond between C7 and C8 atoms) and 3-acetyl Chl a (double bond between C7 and C8 atoms).

Materials and Methods

Apparatus. Electronic absorption and CD spectra were measured with a spectrophotometer (UV-2450, Shimadzu) and a spectropolarimeter (J-820, JASCO), respectively. Fluorescence emission spectra were measured with a fluorescence measurement system (C9920-03G, Hamamatsu Photonics), and apparent fluorescence quantum yields of B850 BChl a were estimated from the emission between 820 and 950 nm using software installed in this system. High-performance liquid chromatography (HPLC) was performed with a pump (LC-20AT, Shimadzu) and a detector (SPD-M20A or SPD-20A, Shimadzu). Resonance Raman spectra were recorded on a Raman microscope (NRS-7100, JASCO).

Materials. LH2 protein was isolated from the cultured cells of the purple photosynthetic bacterium Phs. molischianum DSM120 according to previous reports. B800-depleted LH2 was prepared from native LH2. BChl a was isolated from the cultured cells of Rba. sphaeroides. AcChl a was prepared from BChl a by oxidation with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone. AcChl a was purified on a reverse-phase column SC18-AR-II (10 mm i.d. × 250 mm) with methanol as an eluent before reconstitution into LH2 proteins. A detergent n-dodecyl-β-D-maltoside (DDM) was purchased from Dojindo Laboratories, Co.

Pigment Reconstitution. A solution of B800-depleted LH2 in a mixed buffer of 20 mM Tris and 10 mM succinate containing 0.1% DDM (pH 8.0) was mixed with 1/100 volume of a methanol solution of AcChl a or BChl a, followed by incubation at 35 °C for 2 h in the dark. The sample was concentrated by ultrafiltration using Amicon centric concentrators (30 kDa cutoff, Merk Millipore Ltd.) and was loaded onto a Sephacryl-S200 column (GE Healthcare) in 20 mM Tris buffer containing 0.1% DDM and 150 mM NaCl (pH 8.0). LH2 proteins collected were desalted by ultrafiltration using Amicon centric concentrators (30 kDa cutoff, Merk Millipore Ltd.). LH2 proteins, which are reconstituted with AcChl a and BChl a, are hereafter denoted as AcChl-reconstituted LH2 and BChl-reconstituted LH2, respectively.
The occupancy of AcChl \(a\) in the B800 sites in AcChl-reconstituted LH2 was estimated from electronic absorption spectra of extracted chlorophyllous pigments in the \(Q_y\) region in methanol, as reported elsewhere.\(^{19}\) The occupancy of BChl \(a\) in the B800 sites in BChl-reconstituted LH2 was estimated by comparing \(Q_y\) absorbance of B800 BChl \(a\) in BChl-reconstituted LH2 with that in native LH2.\(^{19}\)

Resonance Raman Spectroscopy. An aliquot of an LH2 solution in 20 mM Tris buffer containing 0.05% DDM (pH 8.0) was deposited on a stainless plate and dried under a stream of \(N_2\) gas. Excitation beam (355 nm) from an Nd:YAG laser was focused onto the sample film through a 40× objective lens. The laser intensity at the sample surface was adjusted to \(\sim 0.4\) mW, and the backscattering from the samples was collected at \(25^\circ C\). Each spectrum was accumulated for 30 s at a single spot to alleviate degradation of the LH2 samples. To improve the signal-to-noise ratio, 27–55 spectra at different points were averaged.

Transient Absorption Spectroscopy. Femtosecond time-resolved transient absorption (TA) experiments were performed with a pair of noncollinear optical parametric amplifiers (NOPA) (TOPAS-white, Light Conversion), pumped by a regeneratively amplified Ti:sapphire laser (Solstice, Spectra-Physics), as light sources. Output of one of the NOPAs was set at either 800 or 695 nm to specifically excite the \(Q_y\) band of BChl \(a\) or AcChl \(a\) in the B800 site for native LH2 or AcChl-reconstituted LH2, respectively, as can be seen in the spectra shown in Figure S1. A prism pair was utilized to precompress the laser pulses, and the pulse duration at the sample position was about 130 ± 3.3 fs (FWHM) for the excitation at 800 nm and about 18.5 ± 0.38 fs (FWHM) at 695 nm, which were measured by the second harmonic generation frequency-resolved optical gating (SHG-FROG) method (Figure S3) and by the self-diffraction frequency-resolved optical gating (SD-FROG) method (Figure S3), respectively. The excitation intensity at the sample position was about \(15 \mu\) W (15 mJ), and the diameter of the focused laser beam was ca. 0.15 mm. By rotating the polarization of the pump pulse by a Berek compensator (Model 5540, New Focus), the polarization between the pump and probe pulses was set at the magic angle. White-light supercontinuum (400–930 nm) was generated by focusing the output of another NOPA centered at 1100 nm into a rotating CaF\(_2\) window (thickness: 2 mm), and it was divided into probe and reference pulses. The probe pulse was focused into the rotating sample cell excited by the pump pulse, and the transmitted light was guided into a multichromometer (MSP1000-V, Unisoku). The reference pulse was directly guided into another multichromometer (MSP1000-V, Unisoku), and the differential absorbance (ΔAbs) of the sample was calculated. The heterodyne-detected optical Kerr effect (HD-OKE) signal between the pump and the probe pulses (Figure S4) was obtained by replacing the sample solution in the rotating cell by neat carbon tetrachloride, and the electronic response signal was utilized to compensate the group velocity dispersion of the TA signal. LH2 proteins were solubilized in 20 mM Tris buffer containing 0.02% DDM (pH 8.0). The optical length of the sample was 2 mm, and the \(Q_y\) band absorbance of B850 BChl \(a\) of the LH2 samples were set at ca. 0.8.

## RESULTS AND DISCUSSION

Electronic Absorption and Fluorescence Emission Spectra. AcChl \(a\) or BChl \(a\) was reconstituted into the B800 cavity of *molischianum*-LH2 by incubation of B800-depleted *molischianum*-LH2 with 20 equivalents of each pigment, followed by purification with size-exclusion chromatography. BChl-reconstituted LH2 exhibited two intense \(Q_y\) bands of B800 and B850 BChl \(a\) at 799 and 846 nm (Figure 2C). These peak positions were identical to those in native LH2 (Figure 2A). AcChl-reconstituted LH2 had no \(Q_y\) band of B800 BChl \(a\) but showed a new absorption band at 690 nm (Figure 2D). This band was ascribed to the \(Q_y\) band of AcChl \(a\) in the B800 site. The \(Q_y\) peak of AcChl \(a\) in the B800 site in *molischianum*-LH2 was shifted to a longer wavelength, by 13 nm (278 cm\(^{-1}\)), than that of its monomer in acetone (Figure S5). This red shift of AcChl \(a\) is smaller than that of BChl \(a\) in the B800 site from its monomeric form in acetone (29 nm, 471 cm\(^{-1}\)). Such a smaller \(Q_y\) red shift is also observed in the reconstitution of AcChl \(a\) into *acidophilus*-LH2.\(^{15}\) These results are rationalized by invoking the changes in the dihedral angles of the 3-acetyl group with the macrocycle plane or the differences in the extent of deformation of the tetrapyrrole macrocycles between AcChl \(a\) and BChl \(a\).\(^{15,41}\)

The \(Q_y\) absorbance of AcChl \(a\) in AcChl-reconstituted LH2 was smaller than that of B800 BChl \(a\) in native LH2 and BChl-reconstituted LH2. This result is partially derived from the smaller transition dipole strength of chlorin-type AcChl \(a\) than BChl \(a\).\(^{19,42,43}\) Another reason is the occupancy of AcChl \(a\) in the eight B800 pockets in *molischianum*-LH2. Extraction of the chlorophyllous pigments from AcChl-reconstituted LH2 indicated that the occupancy was 59 ± 12% in the eight B800 pockets (the average and standard deviation of three samples). In contrast, the occupancy of BChl \(a\) in the B800 pockets of BChl-reconstituted LH2 was estimated to be 107 ± 1% (the average and standard deviation of three samples). Therefore, dehydrogenation of the B-ring from BChl \(a\) (change

![Figure 2](https://dx.doi.org/10.1021/acsomega.0c00152)
from bacteriochlorin to chlorin) impedes binding to the B800 cavity in molischianum-LH2. These results are in sharp contrast to the lack of a change in the affinity to the B800 cavity in acidophilus-LH2 between AcChl a and BChl a.19 The difference can be explained by the pigment orientations in the B800 site; the B-ring in (B)Chl pigments is embedded in the protein matrix and located on the protein surface in molischianum-LH2 and acidophilus-LH2, respectively.5–7,25 Therefore, the effect of the dehydrogenation in the B-ring of BChl a on the binding to the B800 cavity is more significant in molischianum-LH2 than in acidophilus-LH2. In contrast, the pigment binding to the B800 site in sphaeroides-LH2 is affected by the dehydrogenation of the B-ring of BChl a like the case of molischianum-LH2.21 Note that incubation of larger amounts of AcChl a with B800-depleted LH2 gradually increased the occupancy in the B800 sites, but the separation of unbound AcChl a with LH2 by the current size-exclusion chromatography became difficult.

The Q\(_\epsilon\) band of BChl a in AcChl-reconstituted LH2 was positioned at 587 nm (Figure 2D). This Q\(_\epsilon\) position was slightly blue-shifted from the Q\(_\epsilon\) band in native LH2 and BChl-reconstituted LH2 at 590 nm (Figure 2A,C). This blue shift is due to the disappearance of the Q\(_\epsilon\) band of B800 BChl a (Figure 2B) since the Q\(_\epsilon\) band of BChl a in molischianum-LH2 is contributed from both B800 and B850 BChl a, and their Q\(_\epsilon\) positions slightly differed.25,44

The absorption bands of lycopene in AcChl-reconstituted LH2 were positioned at 529 and 493 nm (Figure 2D). These positions were identical to those in native LH2, B800-depleted LH2, and BChl-reconstituted LH2 (Figure 2A–C). These results indicate that the insertion of AcChl a into the B800 site barely affects the electronic properties of lycopene. Note that another band of lycopene at 466 nm, which was detected in the spectra of the other three LH2 proteins, overlapped with the Soret band of AcChl a in the spectrum of AcChl-reconstituted LH2.

AcChl a in the B800 site can transfer excitation energy to B850 BChl a in AcChl-reconstituted LH2, judged by steady-state fluorescence spectroscopy. Selective excitation of AcChl a in the B800 site at 692 nm produced an emission of B850 BChl a around 875 nm (Figure S6B). This corresponds to a B850 emission by the excitation of B800 BChl a at 798 nm in BChl-reconstituted LH2, which is generated by intracomplex EET (Figure S6A). The apparent fluorescence quantum yields of B850 BChl a by the excitation of AcChl a and BChl a in AcChl-reconstituted LH2 and BChl-reconstituted LH2, which are indexes of EET efficiency,20,23,24 were estimated to be 9.5 ± 0.3 and 11 ± 0.5% (the averages and standard deviations of three samples), respectively. The apparent quantum yield of B850 BChl a in AcChl-reconstituted LH2 was slightly smaller than that in BChl-reconstituted LH2.

CD Spectra. Native LH2 exhibited reversed S-shaped and negative CD signals, which were derived from B800 and B850 BChl a, respectively, around 800 and 860 nm (Figure 3A). This spectral feature is characteristic of molischianum-LH2.45,46 In the CD spectra of B800-depleted LH2 and AcChl-reconstituted LH2, the signal of B800 BChl a around 800 nm disappeared (Figure 3B,D). The CD signal of B800 BChl a was completely recovered by the reconstitution of BChl a into the B800 site (Figure 3C). AcChl a in the B800 site showed no CD signal around 700 nm (Figure 3D). The negative CD signal of B850 BChl a in the CD spectrum of AcChl-reconstituted LH2 around 860 nm was analogous to that of the other three LH2 proteins. These results indicate that the insertion of AcChl a into the B800 site barely perturbs the orientation and electronic structures of B850 BChl a.

Resonance Raman Spectra. The interactions of AcChl a in the B800 site with the polypeptides in molischianum-LH2 were scrutinized by resonance Raman spectroscopy (Figure 4). All of the LH2 proteins examined here had intense Raman signals at 1603 cm\(^{-1}\), which were assigned to the stretching modes of the methine bridges of (B)Chl pigments. Native LH2 exhibited the 3-C=C=O and the 13-C=C=O stretching vibrational bands of BChl a at 1635 and 1659 cm\(^{-1}\), respectively (Figure

Figure 3. CD spectra of native LH2 (A), B800-depleted LH2 (B), BChl-reconstituted LH2 (C), and AcChl-reconstituted LH2 (D) in the Q\(_x\) region in 20 mM Tris buffer containing 0.05% n-dodecyl-β-D-maltoside (pH = 8.0). The Q\(_x\) absorbance values of B850 BChl a in the LH2 samples used for the measurements were 0.5.
A difference Raman spectrum between B800-depleted LH2 (Figure 4D) and native LH2 showed the 3-C=O stretching vibrational band of B800 BChl$\alpha$ at 1628 cm$^{-1}$ as a negative signal (Figure 4G). This result indicates that the 3-acetyl group in B800 BChl$\alpha$ is hydrogen-bonded with polypeptides in molischianum-LH2, as revealed in its crystal structure.5 The 13-C=O band of B800 BChl$\alpha$ was observed at 1652 cm$^{-1}$ as a negative signal in the difference spectrum (Figure 4G). This is in line with a previous report on Raman spectroscopy of molischianum-LH2.47 BChl-reconstituted LH2 (Figure 4B) showed the 3-C=O and the 13-C=O stretching vibrational bands of BChl$\alpha$ at almost the same positions (1636 and 1663 cm$^{-1}$) as those of native LH2, indicating that BChl$\alpha$ is properly accommodated in the B800 site. This is supported by few Raman signals in a difference spectrum between BChl-reconstituted LH2 and native LH2 (Figure 4E).

A difference Raman spectrum between AcChl-reconstituted LH2 and native LH2 showed a positive signal at 1640 cm$^{-1}$ with a negative signal at 1625 cm$^{-1}$ (Figure 4F). This pattern was assigned to a higher-frequency shift of the 3-C=O stretching vibrational band of the 3-acetyl group in AcChl$\alpha$ than that in B800 BChl$\alpha$. This shift suggests that the 3-acetyl group in AcChl$\alpha$ is hydrogen-bonded with $\beta'$-Thr23, but the hydrogen bond is weaker than that between B800 BChl$\alpha$ and $\beta'$-Thr23 in native LH2. Since the 3-acetyl group in B800 BChl$\alpha$ is strongly hydrogen-bonded with $\beta'$-Thr23 in native LH2 (hydrogen-bond length, 2.3 A),25 this hydrogen bond is sensitive to a slight change in the orientation of the 3-acetyl group. Therefore, the decrease in the strength of the hydrogen bond between the 3-acetyl group in AcChl$\alpha$ and $\beta'$-Thr23 suggests that the dehydrogenation of the C7=C8 bond produces a small distortion in the orientation of the 3-acetyl group in the B800 cavity. The difference in the 3-C=O stretching vibrational bands between AcChl$\alpha$ and BChl$\alpha$ is qualitatively in line with the smaller red shift of the Qy band in AcChl$\alpha$ by insertion into the B800 cavity compared to BChl$\alpha$ (Figure 2) since the downshift of the 3-C=O stretching mode of BChl$\alpha$ from the monomeric form is correlated with its Qy peak positions in light-harvesting proteins of purple bacteria.28

The 13-C=O stretching vibrational band of the chlorophyllous pigments in AcChl-reconstituted LH2 was detected at 1660 cm$^{-1}$ (Figure 4C), whose position was almost identical to that in native LH2 (Figure 4A). A positive signal in the difference spectrum between AcChl-reconstituted LH2 and native LH2 at 1557 cm$^{-1}$ was assigned to the C7=C8 stretching vibrational band of AcChl$\alpha$ (Figure 4F).

**Energy Transfer Dynamics.** To investigate intracomplex EET, femtosecond TA spectroscopy of native LH2 and AcChl-reconstituted LH2 was conducted. In the TA spectra of native LH2 (Figure 5A) excited at 800 nm, a negative band with a minimum at 800 nm appeared immediately after the excitation. A similar immediate appearance of a negative band at 695 nm was also observed in the TA spectra of AcChl-reconstituted LH2 (Figure 5B) excited at 695 nm. These bands can be assigned to the superposition of the ground-state bleach (GSB) and the stimulated emission (SE) of the energy donors. By increasing the delay time between the pump and probe pulses, these bands decayed and new positive and negative bands at 830 and 855 nm appeared simultaneously, which indicates EET from the donors to the acceptor B850. The time evolution of the spectra was much slower for AcChl-reconstituted LH2, indicating slower EET compared to native LH2.

Global analysis was applied to the time dependence of the differential absorbance ($\Delta$Abs) at various wavelengths (Figure 6), and decay-associated spectra (DAS) were obtained (Figures 7 and 8). Two DAS components were applied to represent the donor and the acceptor of the EET, and any intramolecular energy relaxation process (spectral shift) was ignored. As seen in Figure 6, the experimental and fitting...
results are in good agreement, confirming the validity of this model. The DAS components A (red curves in Figures 7 and 8) with shorter lifetimes represent the decay and the rise of the EET donor (B800 BChl a) and acceptor (B850 BChl a), and the DAS components B (blue curves in Figures 7 and 8) with longer lifetimes represent the decay of the excited state of B850 BChl a. The lifetime for the shorter DAS components was 990 ± 2.8 fs for native LH2 (Figure 7), but it extended to 5.0 ± 0.08 ps for AcChl-reconstituted LH2 (Figure 8). The DAS component A in Figure 7 exhibits a negative band at 800 nm, suggesting the decrease of the mixed GSB/SE band of B800 BChl a, while a weak negative band at 695 nm in the DAS component A in Figure 8 corresponds to the decrease of the mixed GSB/SE band of reconstituted AcChl a. Negative and positive bands at 830 and 855 nm in components A in both Figures 7 and 8 represent the increase of the positive excited-state absorption band and the negative GSB/SE band of B850 BChl a, respectively. The lifetime for component A in Figure 7 is consistent with the EET time constant previously reported for native molischianum-LH2 at room temperature.45,49 The slower decay of component A in Figure 8 suggests that intracomplex EET in AcChl-reconstituted LH2 is about 5-fold slower than that in native LH2.

It was previously reported that AcChl a in the B800 site of acidophilus-LH2 and sphaeroides-LH2 exhibits EET to B850 BChl a with a time constant of about 5 ps,15,21 which is comparable to the currently reported result. The major reason for the slower EET for AcChl-reconstituted LH2 is the decrease of the spectral overlap between the EET donor and the acceptor. This study thus demonstrates that the spectral overlap between the cyclic tetrapyrroles in the B800 site and B850 BChl a is generally responsible for EET dynamics in LH2 proteins.

AcChl-reconstituted LH2 exhibited homogeneous EET dynamics from the energy-donating semiartificial pigment (AcChl a) to the energy accepter (B850 BChl a) due to the accommodation of AcChl a in the well-defined environment. The current homogeneous EET dynamics is in sharp contrast to the multicomponent EET in LH2 covalently attached with artificial chromophores.32 Therefore, the reconstitution of artificial chromophores into the B800 site in LH2 is advantageous for development of biohybrid light-harvesting proteins.

■ CONCLUSIONS

AcChl a was successfully reconstituted into the B800 site in molischianum-LH2. The red shift of the Q, band of AcChl a induced by insertion into the B800 cavity was smaller than that of BChl a in molischianum-LH2, suggesting that the dihedral angle of the 3-acetyl group with the macrocycle plane or the deformation of the macrocycle of AcChl a in the B800 cavity differed from those of BChl a in molischianum-LH2. Resonance Raman spectroscopy demonstrated that the 3-acetyl group in AcChl a in the B800 site is hydrogen-bonded with β′-Thr23 in molischianum-LH2. This hydrogen-bonding pattern is consistent with B800 BChl a in native LH2, although the hydrogen bond between the 3-acetyl group and β′-Thr23 is weakened by the dehydrogenation of the B-ring from BChl a (7,8,17,18-tetrahydrophyrin → 17,18-dihydroporphyrin). CD spectroscopy indicates that AcChl a is accommodated in molischianum-LH2 without perturbation of the local protein structure.

AcChl a in the B800 site functioned as an energy donor to B850 BChl a in molischianum-LH2. The energy transfer rate from AcChl a in the B800 site to B850 BChl a was about 5-fold slower than that from B800 BChl a. The slow kinetics is likely due to the decreased spectral overlap between an emission band of AcChl a and B850 BChl a. This study demonstrates that the replacement of B800 BChl a with AcChl a enables molischianum-LH2 to collect red light, which is not captured by LH2 despite red wavelengths being most abundant at the
surface of Earth. Insertion of modified pigments into the B800 site in molischianum-LH2 will thus be useful not only for elucidation of the mechanisms of pigment binding and intracomplex EET in LH2 proteins but also for engineering the photofunctions of LH2 proteins toward the control of photosynthetic activities in purple bacteria.

## ASSOCIATED CONTENT

* Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00152.

Supplementary data on TA spectroscopy (Figures S1–S4); electronic absorption spectrum of AcChl a (Figure S5); and fluorescence emission spectra of reconstituted LH2 (Figure S6) (PDF)

## Accession Codes

The UniProt accession numbers for *molischianum*-LH2 are P97253 and P95673.

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### Notes

The authors declare no competing financial interest.

## ABBREVIATIONS

AcChl, 3-acetyl Chl; BChl, bacteriochlorophyll; *Pbs*, *Phaeospirillum*; *Rbl*, *Rhodoblastus*; *Rba.*, *Rhodobacter*; *molischianum*-LH2, LH2 protein derived from *Pbs. molischianum*; *acidiophilus*-LH2, LH2 protein derived from *Rbl. acidophilus*; *sphaeroideas*-LH2, LH2 protein derived from *Rba. sphaeroides*; B800-depleted LH2, LH2 protein that has no B800 BChl a; AcChl-reconstituted LH2, LH2 that is reconstituted with 3-acetyl Chl a into the B800 site; BChl-reconstituted LH2, LH2 that is reconstituted with BChl a into the B800 site; DDM, n-dodecyl-

β-2-maltoside; DAS, decay-associated spectra; EET, excitation energy transfer; GSB, ground-state bleach; SE, stimulated emission; HD-OKE, heterodyne-detected optical Kerr effect; NOPA, noncollinear optical parametric amplifier; TA, transient absorption

## REFERENCES

1. Scheer, H. An Overview of Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications. In *Chlorophylls and Bacteriochlorophylls: Biochemistry, Biophysics, Functions and Applications*; Grimm, B.; Pora, R. J.; Rüdiger, W.; Scheer, H., Eds.; Springer: Dordrecht, 2006; pp 1–26.

2. Croce, R. van Amerongen, H. Natural strategies for photosynthetic light harvesting. *Nat. Chem. Biol.* 2014, 10, 492–501.

3. Saer, R. G.; Blankenship, R. E. Light-harvesting in phototrophic bacteria: structure and function. *Bioch. J. 2017,* 474, 2107–2131.

4. Tamiaki, H.; Kunieda, M. Photochemistry of Chlorophylls and their Analogs. In *Handbook of Porphyryrin Science* Kadish, K. M.; Smith, K. M.; Guilard, R., Eds.; World Scientific: Singapore, 2011; Vol 11, pp 223–290.

5. Koepke, J.; Hu, X.; Muenke, C.; Schulten, K.; Michel, H. The crystal structure of the light-harvesting complex II (B800–850) from *Rhodopseudomonas molischianum*. Structure 1996, 4, 581–597.

6. McDermott, G.; Prince, S. M.; Freer, A. A.; Hathornthwaitie-Lawless, A. M.; Papiz, M. Z.; Cogdell, R. J.; Issacs, N. W. Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria. *Nature 1995,* 374, 517–521.

7. Papiz, M. Z.; Prince, S. M.; Howard, T.; Cogdell, R. J.; Issacs, N. W. The structure and thermal motion of the B800–B850 LH2 complex from *Rps. acidophila* at 2.0 Å resolution and 100 K: new structural features and functionally relevant motions. *J. Mol. Biol. 2003,* 326, 1523–1538.

8. Sundström, V.; Pullerits, T.; van Grondelle, R. Photosynthetic light-harvesting: reconciling dynamics and structure of purple bacterial LH2 reveals function of photosynthetic unit. *J. Phys. Chem. B 1999,* 103, 2327–2346.

9. Cogdell, R. J.; Gall, A.; Köhler, J. The architecture and function of the light-harvesting apparatus of purple bacteria: from single molecules to in vivo membranes. *Q. Rev. Biophys. 2006,* 39, 227–324.

10. Scholes, G. D.; Fleming, G. R.; Olaya-Castro, A.; van Grondelle, R. Lessons from nature about solar light harvesting. *Nat. Chem. 2011,* 3, 763–774.

11. Georgakopoulos, S.; Frese, R. N.; Johnson, E.; Koolhaas, C.; Cogdell, R. J.; van Grondelle, R.; van der Zwan, G. Absorption and CD spectroscopy and modeling of various LH2 complexes from purple bacteria. *Biophys. J. 2002,* 82, 2184–2197.

12. Bandilla, M.; Ucker, B.; Ram, M.; Simonin, I.; Gelhaye, E.; McDermott, G.; Cogdell, R. J.; Scheer, H. Reconstitution of the B800 bacteriochlorophylls in the peripheral light harvesting complex B800–850 of *Rhodobacter sphaeroides* 2.4.1 with BChl a and modified (bacterio)-chlorophylls. *Biochim. Biophys. Acta 1998,* 1364, 390–402.

13. Fraser, N. J.; Dominy, P. J.; Ucker, B.; Simonin, I.; Scheer, H.; Cogdell, R. J. Selective release, removal, and reconstitution of bacteriochlorophyll a molecules into the B800 sites of LH2 complexes from *Rhodopseudomonas acidophila* 10050. *Biochemistry 1999,* 38, 9684–9692.

14. Gall, A.; Fraser, N. J.; Bellissent-Funel, M.-C.; Scheer, H.; Robert, B.; Cogdell, R. J. Bacteriochlorin-protein interactions in native B800–B850, B800 deficient and B800–BChla-reconstituted complexes from *Rhodopseudomonas acidophila*, strain 10050. *FEBS Lett. 1999,* 440, 269–272.

15. Herek, J. L.; Fraser, N. J.; Pullerits, T.; Martinsson, P.; Polivka, T.; Scheer, H.; Cogdell, R. J.; Sundström, V. B800–B850 energy transfer mechanism in bacterial LH2 complexes investigated by B800 pigment exchange. *Biophys. J. 2000,* 78, 2590–2596.

16. Gall, A.; Robert, B.; Cogdell, R. J.; Bellissent-Funel, M.-C.; Fraser, N. J. Probing the binding sites of exchanged chlorohyll a in
Reconstitution of chlorophyll b into the bacterial photosynthetic light-harvesting protein LH2. Chem. Lett. 2018, 47, 1071–1074.

(21) Swainsbury, D. J. K.; Faries, K. M.; Niedzwiedzki, D. M.; Martin, E. C.; Flinders, A. J.; Canniffe, D. P.; Shen, G.; Bryant, D. A.; Kiraider, C.; Holten, D.; Hunter, C. N. Engineering of B800 bacteriochlorophyll binding site specificity in the Rhodobacter sphaeroides LH2 antenna. Biochim. Biophys. Acta 2019, 1860, 209–223.

(22) Niedzwiedzki, D. M.; Swainsbury, D. J. K.; Hunter, C. N. Carotenoid-to-(bacterio)chlorophyll energy transfer in LH2 antenna complexes from Rba. sphaeroides reconstituted with non-native (bacterio)chlorophylls. Photosynth. Res. 2019, DOI: 10.1007/s11120-019-00661-6.

(23) Saga, Y.; Kawano, K.; Otsuka, Y.; Imanishi, M.; Kimura, Y.; Matsu, S.; Asakawa, H. Selective oxidation of B800 bacteriochlorophyll a in photosynthetic light-harvesting protein LH2. Sci. Rep. 2019, 9, No. 3636.

(24) Saga, Y.; Yamashita, M.; Nakagawa, S. In situ conversion of chlorophyll b reconstituted into photosynthetic protein LH2. Chem. Lett. 2019, 48, 1270–1273.

(25) Saga, Y.; Hirota, K.; Matsu, S.; Asakawa, H.; Isikita, H.; Saito, K. Selective removal of B800 bacteriochlorophyll a from light-harvesting complex 2 of the purple photosynthetic bacterium Phaeospirillum molischianum. Biochemistry 2018, 57, 3075–3083.

(26) Nakamura, A.; Mizoguchi, S.; Yoshida, E.; Kato, Y.; Watanabe, T. Light-induced charge separation in photosystem I can be sensitized by an artificial fluorescent dye covalently linked to the photosystem I complex surfaces. Chem. Lett. 2005, 34, 1472–1473.

(27) Springer, J. W.; Parkes-Loach, P. S.; Reddy, K. R.; Krayev, M.; Jiao, J.; Lee, G. M.; Niedzwiedzki, D. M.; Harris, M. A.; Kiraider, C.; Bocian, D. F.; Lindsay, J. S.; Holten, D.; Loach, P. A. Biobhybrid photosynthetic antenna complexes for enhanced light-harvesting. J. Am. Chem. Soc. 2012, 134, 4589–4599.

(28) Reddy, K. R.; Jiang, J.; Krayev, M.; Harris, M. A.; Springer, J. W.; Yang, E.; Jiao, J.; Niedzwiedzki, D. M.; Pandithaviana, D.; Parkes-Loach, P. S.; Kiraider, C.; Loach, P. A.; Bocian, D. F.; Holten, D.; Lindsay, J. S. Palette of lipophilic biocompatible bacteriochlorins for construction of biobhybrid light-harvesting architectures. Chem. Sci. 2013, 4, 2036–2053.

(29) Harris, M. A.; Parkes-Loach, P. S.; Springer, J. W.; Jiang, J.; Martin, E. C.; Qian, P.; Jiao, J.; Niedzwiedzki, D. M.; Kiraider, C.; Olsen, J. D.; Bocian, D. F.; Holten, D.; Hunter, C. N.; Lindsay, J. S.; Loach, P. A. Integration of multiple chromophores with native photosynthetic antennas to enhance solar energy capture and delivery. Chem. Sci. 2011, 2, 3924–3933.

(30) Harris, M. A.; Jiang, J.; Niedzwiedzki, D. M.; Jiao, J.; Taniguchi, M.; Kiraider, C.; Loach, P. A.; Bocian, D. F.; Lindsay, J. S.; Holten, D.; Parkes-Loach, P. S. Versatile design of biobhybrid light-harvesting architectures to tune location, density, and spectral coverage of attached synthetic chromophores for enhanced energy capture. Photosynth. Res. 2014, 121, 35–48.

(31) Dutta, P. K.; Lin, S.; Loskutov, A.; Levenberg, S.; Jun, D.; Saer, R.; Beatty, J. T.; Liu, Y.; Yan, H.; Woodbury, N. W. Reengineering the optical absorption cross-section of photosynthetic reaction centers. J. Am. Chem. Soc. 2014, 136, 4599–4604.

(32) Yoneda, Y.; Noji, T.; Katayama, T.; Mizutani, N.; Kosumi, D.; Nango, M.; Miyasaka, H.; Itoh, S.; Nagasawa, Y.; Dewa, T. Extension of light-harvesting ability of photosynthetic light-harvesting complex 2 (LH2) through ultrafast energy transfer from covalently attached artificial chromophores. J. Am. Chem. Soc. 2015, 137, 13121–13129.

(33) Yoneda, Y.; Kato, D.; Kondo, M.; Nagashima, K. V. P.; Miyasaka, H.; Nagasawa, Y.; Dewa, T. Sequential energy transfer driven by monoeponential dynamics in a biohybrid light-harvesting complex 2 (LH2). Photosynth. Res. 2020, 143, 115–128.

(34) Kondo, M.; lida, K.; Dewa, T.; Tanaka, H.; Ogawa, T.; Nagashima, S.; Nagashima, K. V. P.; Shimada, K.; Hashimoto, H.; Gardiner, A. T.; Cogdell, R. J.; Nango, M. Photocurrent and electronic activities of oriented-His-tagged photosynthetic light-harvesting/reaction center complex assembled onto a gold electrode. Biomacromolecules 2012, 13, 432–438.

(35) Sumino, A.; Dewa, T.; Sasaki, N.; Kondo, M.; Nango, M. Electron conduction and photocurrent generation of a light-harvesting/reaction center complex in lipid membrane environments. J. Phys. Chem. Lett. 2013, 4, 1087–1092.

(36) Ravi, S. K.; Yu, Z.; Swainsbury, D. J. K.; Ouyang, J.; Jones, M. R.; Tan, S. C. Enhanced output from biobhybrid photoelectrochemical transparent tandem cells integrating photosynthetic proteins genetically modified for expanded solar energy harvesting. Adv. Energy Mater. 2017, 7, No. 1601821.

(37) Saga, Y.; Hirota, K. Determination of the molar extinction coefficients of the B800 and B850 absorption bands in light-harvesting complexes 2 derived from three purple photosynthetic bacteria Rhodoblastus acidophilus, Rhodobacter sphaeroides, and Phaeospirillum molischianum by extraction of bacteriochlorophyll a. Anal. Sci. 2016, 32, 801–804.

(38) Saga, Y.; Miura, R.; Sadoaka, K.; Hirai, Y. Kinetic analysis of demetallation of synthetic zinc cyclic tetrapyroles possessing an acetyl group at the 3-position: effects of tetrapyrole structures and peripheral substitution. J. Phys. Chem. B 2011, 115, 11757–11762.

(39) Saga, Y.; Hirota, K.; Harada, J.; Tamaki, H. In vitro enzymatic activities of bacteriochlorophyll a synthase derived from the green sulfur photosynthetic bacterium Chlorobaculum tepidum. Biochemistry 2015, 54, 4998–5005.

(40) Smith, J. R. L.; Calvin, M. Studies on the chemical and photochemical oxidation of bacteriochlorophyll. J. Am. Chem. Soc. 1966, 88, 4500–4506.

(41) Cogdell, R. J.; Howard, T. D.; Isaacs, N. W.; McLuskey, K.; Gardiner, A. T. Structural factors which control the position of the Qy absorption band of bacteriochlorophyll a in purple bacterial antenna complexes. Photosynth. Res. 2002, 74, 135–141.

(42) Knox, R. S. Dipole and oscillator strengths of chromophores in solution. Photochem. Photobiol. 2003, 77, 492–496.

(43) Knox, R. S.; Spring, B. Q. Dipole strengths in the chlorophylls. Photochem. Photobiol. 2003, 77, 501–502.

(44) Thyrhaug, E.; Lincoln, C. N.; Branchi, F.; Cerullo, G.; Perlik, V.; Sanda, F.; Lokstein, H.; Hauer, J. Carotenoid-to-bacteriochlorophyll energy transfer through vibronic coupling in LH2 from Phaeospirillum molischianum. Photosynth. Res. 2018, 135, 45–54.

(45) Ihalainen, J. A.; Linnanto, J.; Myllyperkio, P.; van Stolkum, I. H. M.; Ücker, B.; Scheer, H.; Korppi-Tommola, J. E. I. Energy transfer in LH2 of Rhodospirillum molischianum studied by subpicosecond spectroscopy and configuration interaction excitation calculations. J. Phys. Chem. B 2001, 105, 9849–9856.

(46) Todd, J. B.; Parkes-Loach, P. S.; Leykam, J. F.; Loach, P. A. In vitro reconstitution of the core and peripheral light-harvesting complexes of Rhodospirillum molischianum from separately isolated components. Biochemistry 1998, 37, 17458–17468.

(47) Sturgis, J. N.; Robert, B. Pigment-binding site and electronic properties in light-harvesting proteins of purple bacteria. J. Phys. Chem. B 1997, 101, 7227–7231.
(48) Germeroth, L.; Lottspeich, F.; Robert, B.; Michel, M. Unexpected similarities of the B800-B850 light-harvesting complex from *Rhodospirillum molischianum* to the B870 light-harvesting complexes from other purple photosynthetic bacteria. *Biochemistry* 1993, 32, 5615–5621.

(49) Salverda, J. M.; van Mourik, F.; van der Zwan, G.; van Grondelle, R. Energy transfer in the B800 rings of the peripheral bacterial light-harvesting complexes of *Rhodopseudomonas acidophila* and *Rhodospirillum molischianum* studied with photon echo techniques. *J. Phys. Chem. B* 2000, 104, 11395–11408.