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Invited paper

Insertion of chlorophyll *a* derivatives into the binding sites of B800 bacteriochlorophyll *a* in light-harvesting complex 2 from the purple photosynthetic bacterium *Rhodoblastus acidophilus*

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ABSTRACT

The LH2 protein has two types of bacteriochlorophyll (BChl) *a* termed B800 and B850 BChl *a*. Substitution of B800 BChl *a* with chlorophyll (Chl) *a* derivatives in this protein has considerable attention from the viewpoints of elucidation of the energy transfer mechanism and developments of artificial photosynthetic devices based on the LH2 structure. Herein we examine reconstitution properties of Chl *a* and 13^2-demethoxycarbonyl Chl *a* (pyroChl *a*) into the B800 binding sites in LH2 from the purple photosynthetic bacterium *Rhodoblastus* (*Rbl.*) *acidophilus*. Both the Chl *a* derivatives were successfully inserted into the B800 binding sites in the LH2 protein whose B800 BChl *a* was removed (B800-free LH2), and the energy transfer from these derivatives to B850 BChl *a* was observed in the reconstituted LH2 proteins. Extraction of chlorophyllous pigments from the reconstituted LH2 proteins allows us to estimate the occupancy of Chl *a* and pyroChl *a* in the nine B800 binding sites from the molar ratio of (pyro)Chl *a* to residual B850 BChl *a*: the occupancy of Chl *a* was 72% and was 1.2-times larger than that of pyroChl *a*. These results indicate that Chl *a* and pyroChl *a* can be accommodated in the B800 binding sites in the LH2 protein derived from this bacterium, but the affinity of pyroChl *a* would be slightly smaller than that of Chl *a*. The binding properties of Chl *a* derivatives into the B800 binding sites in LH2 will be useful for manipulation of light-harvesting abilities in photosynthetic antenna proteins.

1. Introduction

Natural photosynthesis is an excellent light-conversion system on earth. The efficient collection of sunlight and its conversion to chemical energy in the photosynthetic systems is based on the arrangement of functional pigments such as chlorophyll (Chl), bacteriochlorophyll (BChl), and carotenoid molecules. These photosynthetic pigments are generally embedded in the protein matrixes [1]. Such pigment organization in photosynthetic proteins has attracted much attention, not only because of its fundamental importance in photobiological chemistry but also because of developments of artificial photosynthetic nanodevices [2–7].

The light-harvesting complex 2 (LH2) is a peripheral antenna protein in purple photosynthetic bacteria. In the LH2 proteins, BChl *a* molecules (Fig. 1A) are organized in a circular form. Crystallographic analysis demonstrates that the LH2 proteins derived from *Rhodoblastus* (*Rbl.*) *acidophilus* and *Phaeospirillum molischianum* are composed of nine and eight units [8–10]. BChl *a* molecules in the LH2 proteins are classified into two groups: one is called B800 BChl *a*, which exhibits the Q_y absorption band around 800 nm and the other is B850 BChl *a* possessing the Q_y band around 850 nm. The tetrapyrrole macrocyclic planes of B800 and B850 BChl *a* are parallel and perpendicular to the photosynthetic membrane, respectively. B800 BChl *a* is monomeric in the LH2 proteins, whereas B850 BChl *a* molecules are present as a dimeric form in one unit of this proteins and are excitonically coupled in the circular arrangement.

In the LH2 protein, the excitation energy is transferred from B800 to B850 BChl *a* in a high efficiency. The energy transfer between the two types of BChl *a* in the LH2 protein is of interest in the research area of photobiological chemistry, and its mechanism has extensively been studied [2,3,5,6]. Substitution of B800 BChl *a* with other chlorophyllous pigments has been recognized as one of the powerful methodologies for investigation of the energy

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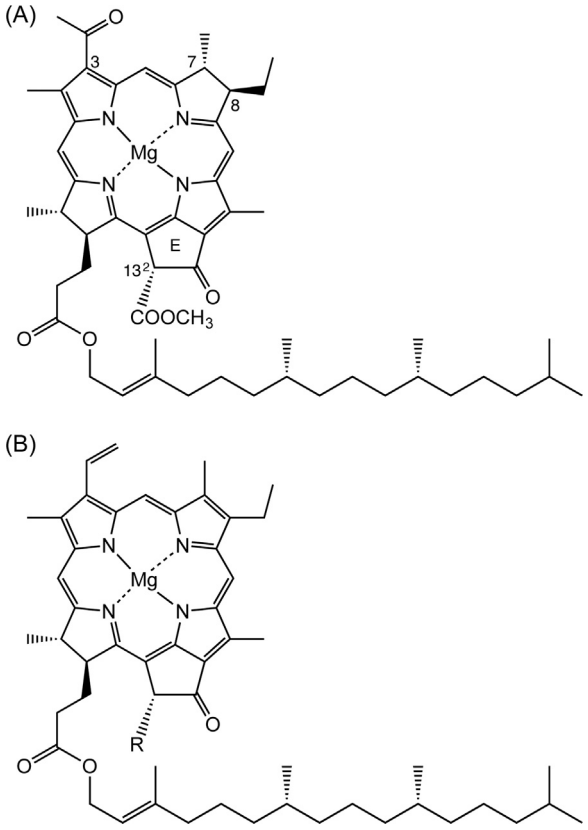

Fig. 1. (A) Molecular structures of BChl *a* (A) and Chl *a* derivatives used in this study (B). Chl *a*: R = $COOCH_3$, pyroChl *a*, R = H.

transfer mechanism in LH2. Additionally, such pigment substitution will be useful for developments of artificial photosynthetic nanodevices based on the LH2 protein structure. From these viewpoints, utilization of various chlorophyllous pigments will be desirable for substitution of B800 BChl *a*. It has been reported that some (B)Chl *a* derivatives, whose central metal, 3-substitutent, exocyclic E-ring, and macrocyclic skeleton are modified, are successfully reconstituted into the B800 binding sites in the LH2 proteins [11–15]. In spite of the extensive studies, however, no direct information is available on the amounts of the pigments inserted into the B800 binding sites in LH2: the occupancy of exogenous pigments in these sites were roughly estimated by electronic absorption spectra of reconstituted LH2 proteins itself [11]. In the first point of this paper, we directly estimate the molar ratio of Chl *a* molecules to residual B850 BChl *a* that were present in reconstituted LH2 by pigment extraction, and discuss the relationship between the occupancy of Chl *a* and its Q_y absorbance in the reconstituted proteins.

The structural requirements of (B)Chl derivatives for insertion into the B800 binding sites in LH2 are of considerable interest. Bandilla et al. reported that the exocyclic E-ring including the 13^2-methoxycarbonyl group in BChl *a* was important in its binding to the B800 binding sites in the LH2 protein derived from *Rhodobacter* (*Rba.*) *sphaeroides* [11]. However, it remains somewhat unclear whether the role of the 13^2-methoxycarbonyl group in (B)Chl derivatives in their reconstitution into the B800 binding sites is referred to LH2 from other purple bacteria. Generally, (B)Chl derivatives possessing the 13^2-methoxycarbonyl group are unstable, namely the exocyclic E-ring is transformed by reactions called allomerization [16–19]. In this aspect, (B)Chl derivatives lacking this group called pyro(B)Chl derivatives have been widely utilized to construct model systems mimicking photosynthetic supramolecules [20–25]. Therefore, it will be a clue to development of artificial photosynthetic nanodevices based on the LH2 structure whether pyro(B)Chl derivatives are acceptable for the B800 binding sites in LH2 derived from some species of purple bacteria. In the second point of this paper, we examine the effect of the 13^2-methoxycarbonyl group in Chl *a* derivatives upon reconstitution into the B800 binding sites in LH2 derived from *Rbl. acidophilus* using Chl *a* and pyroChl *a* (Fig. 1B).

2. Experimental

2.1. Apparatus

Visible absorption spectra were measured with a Shimadzu UV-2450 spectrophotometer. Circular dichroism (CD) spectra were measured with a JASCO J-820 spectropolarimeter. Fluorescence emission spectra were measured with a Hamamatsu C9920-03G fluorescence measurement system. Apparent fluorescence quantum yields of B850 BChl *a* were calculated from the emissions between 830 and 940 nm by a software installed in a Hamamatsu C9920-03G fluorescence measurement system. High-performance liquid chromatography (HPLC) was performed with a Shimadzu LC-20AT pump and an SPD-M20A detector. Lyophilization was carried out with a Tokyo Rikakikai FDU-1200 freeze dryer.

2.2. Materials

The LH2 protein was isolated from a purple photosynthetic bacterium *Rbl. acidophilus* DSM137 [26,27]. B800 BChl *a* was removed from the LH2 protein under the acidic conditions, followed by purification with cation-exchange column chromatography in the same procedure as a previous report [27]. Chl *a* was isolated from a cyanobacterium *Spirulina geitleri* [28,29]. The 13^2-methoxycarbonyl group in Chl *a* was eliminated by pyrolysis of Chl *a* in pyridine to obtain pyroChl *a* [30,31]. Chl *a* and pyroChl *a* were purified by high-performance liquid chromatography using a reverse-phase column Cosmosil $5C_{18}$-AR-II (10 mm i.d. × 250 mm) before reconstitution into LH2.

2.3. Reconstitution of Chl a derivatives

A 30-μL acetone solution of Chl *a* or methanol solution of pyroChl *a* was mixed with a 570-μL solution of LH2 that lacked B800 BChl *a* (denoted as B800-free LH2) in 20 mM Tris-HCl buffer (pH = 8.0) containing 0.1% *n*-dodecyl-*β*-D-maltoside, followed by incubation for 2 h at 4 °C in the dark. The molar ratio of B800-free LH2 and (pyro)Chl *a* was 1:50 in the mixed solution. Then, the LH2 protein was separated from unbound pigments by anion-exchange column chromatography using Whatman DE52 resin. Fractions containing LH2, judged from their electronic absorption spectra, were collected and were analyzed by electronic absorption, fluorescence emission, and CD spectroscopy.

2.4. Pigment extraction from reconstituted LH2

Chlorophyllous pigments were extracted from lyophilized reconstituted LH2 as follows [26]. Reconstituted LH2 proteins were frozen using liquid nitrogen, and dried under the reduced pressure in the dark. Chlorophyllous pigments were rapidly extracted with 3-mL methanol, followed by measurements of electronic absorption spectra immediately. The ratio of Chl *a* and BChl *a* in methanol was determined by using their molecular extinction coefficient 7.14×10^4 and $6.00 \times 10^4\ M^{-1}\ cm^{-1}$ at the Q_y peaks [32]. The molar extinction coefficients of Chl *a* was also applied to estimate the molar ratio of pyroChl *a* against BChl *a*.

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