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Reconstitution and Organization of Photosynthetic Antenna Protein Complex Bearing Functional Hydrophilic Domains

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Bacterial photosynthetic antenna polypeptide (LH1-α) was synthesized as a water-soluble fusion protein with maltose-binding protein (MBP) and a His-tag portion (MBP–rubα-YH) using an *E. coli* expression system. Reconstitution experiments indicated that LH1-type complexes with pigments were successfully formed, regardless of the presence of a large hydrophilic MBP portion. The reconstituted complex was immobilized onto a Au electrode via a His-tag–Ni–NTA interaction. When the complex was incorporated into a planar lipid bilayer, protruding MBP portions were clearly observed by AFM.

Antenna complexes of purple photosynthetic bacteria, LH1 and LH2 are quaternary supramolecular complexes composed of a set of hydrophobic peptides (LH-α and -β) and pigments (bacteriochlorophylls (BChl) and carotenoids) that efficiently harvest light-energy.[1–4] Such naturally occurring biophotonic complexes have recently received a great deal of attention as optical applications. Recent attempts resulted in LH2 molecules being organized onto a solid substrate through self-assembled monolayers.[5,6] We previously assembled LH1, reaction center (RC), and LH1-containing RC, LH1-RC complexes onto solid supports such as ITO and Au electrodes.[7] Reconstituted LH1-type complexes are also potential candidates for this purpose.[8] Reconstitution methodology affords a wide variety of artificial LH1-type complexes via substitution of the constituents, pigments and polypeptides depending on the architecture of quaternary supramolecular complexes, which may allow light absorption/emission and energy-transfer properties to be tuned. We recently reported that a 1α-type hydrophobic polypeptide, LH1-β polypeptide (*Rhodobacter* (*Rba.*) *sphaeroides*), was successfully expressed as a water-soluble fusion protein with maltose-binding protein (MBP) and that the fusion protein forms a homologous (β/β) subunit-type complex with zinc-substituted BChl *a* (Zn–BChl *a*), regardless of the presence of the large MBP portion on the N-terminal of the LH1-β polypeptide.[9] Such a methodology using gene-engineered mutagenesis prompted us to develop a strategy for the assembly of reconstituted LH1-type complex on a substrate with a defined orientation. Specifically, we designed an LH1-α polypeptide (*Rhodospirillum* (*Rsp.*) *rubrum*) bearing MBP and His-tag domains on the N- and C-terminal regions, respectively (MBP–rubα-YH, Figure 1a). The C-terminal His-tag portion can bind to a Ni–NTA-modified surface to direct the protein to a defined direction. The N-terminal MBP portion is eventually oriented to the opposite direction as a result of reconstitution with the counterpart polypeptide LH1-β (*Rsp. rubrum*) and pigments and immobilization on the substrate. The hydrophilic MBP portion can also be expected to be a landmark (a large hydrophilic MBP) that enables direct observation by atomic force microscopic (AFM) analysis when incorporated into a lipid bilayer.

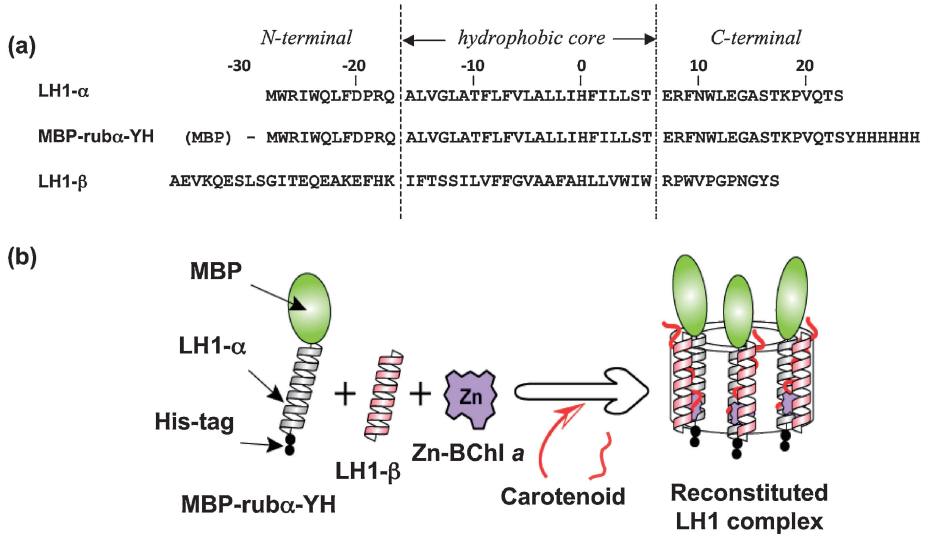

Figure 1. (a) Amino acid sequences of native *Rsp. rubrum* LH1-α, LH1-β peptides, and MBP–rubα-YH. (b) Reconstitution of the LH1 complex using MBP–rubα-YH, LH1-β, Zn–BChl *a*, and a carotenoid (spirilloxanthin, spx or spheroidene, sphe).

In this study, we prepared MBP–rubα-YH to reconstitute an LH1-type complex with LH1-β, Zn–BChl *a*, and carotenoids (spirilloxanthin, spx; spheroidene, sphe; the chemical structures are shown in Figure S1[19]) as shown in Figure 1b. The reconstituted complexes could be successfully assembled onto a Ni–NTA-modified Au substrate. When the reconstituted complex was incorporated into a planar lipid bilayer, the assembled MBP portion was clearly observed by atomic force microscopy as a protrusion from the lipid bilayer.

To synthesize MBP–rubα-YH, the target mutant gene was inserted into a plasmid expression vector, after which the generated construct was expressed in *E. coli* BL21(DE3)pLysS. The expressed protein was subsequently purified by amylose resin column chromatography. Despite the hydrophobic nature of the LH1-α peptide, the MBP–rubα-YH was obtained in good yield (ca. 150 mg per 1 L of medium culture) owing to the increase in water solubility induced by MBP fusion. The purified MBP–rubα-YH was analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (Figure S2[19]) and MALDI-TOF-MS (Figure S3[19]). Reconstitution experiments were conducted according to previously reported procedures.[9–11] Reconstituted complexes were purified using ion-exchange column chromatography and subsequent sucrose density-gradient ultracentrifugation.

The results of the reconstitution experiments are summarized in Table 1 as the λ_{max} of the absorption spectra of reconstituted sample solutions. In the ternary reconstitution system (without carotenoids), the Q_y band of Zn–BChl *a* with MBP–rubα-YH/LH1-β (Entry 2) was identical to that of the reconstituted product with the native LH1-α/LH1-β (Entry 1). The observed Q_y bands of Zn–BChl *a* are indicative of the LH1-type complex.[13] These results indicate that MBP–rubα-YH forms an LH1-type complex, regardless of the large hydrophilic MBP and His-tag portions. In the presence of a carotenoid (either spx or sphe), the Q_y band slightly red-shifted (864 and 865 nm) (Entries 4 and 6). Such a spectral shift is characteristic of incorporation of carotenoid molecules into the LH1-type complex (Entries 3 and 5).[14] Again, the Q_y bands of the reconstituted complexes containing MBP–rubα-YH were identical to those containing LH1-α (Entries 3–6). The absorption bands were also consistent with that of the chromatophore of *Acidiphilium* (*Acp.*) *rubrum*, which may contain Zn–BChl *a*-enriched LH1,[12] implying that the reconstituted complexes are analogous to the native LH1 complex. Spirilloxanthin (spx) and spheroidene (sphe) are known to be major components of carotenoids in LH1-RC complexes from *Rsp. rubrum* (90%) and *Rba. sphaeroides* (85%).[15] In a previous report, we showed that LH1-α and -β polypeptides from *Rsp. rubrum* could incorporate these carotenoids through reconstitution experiments with Zn–BChl *a*.[11] The observed spectral data are in good agreement with the previous data from the reconstitution experiments, indicating that the MBP–rubα-YH possesses the ability to form a LH1-type complex similar to native LH1-α polypeptide. Other spectral data observed for MBP–rubα-YH-based reconstituted complexes such as the absorption bands of Q_x of Zn–BChl *a* and triplet bands of spx and sphe were also consistent with the corresponding LH1-α-based complexes.

Immobilization of the quaternary reconstituted complex (MBP–rubα-YH/LH1-β/Zn–BChl *a*/sphe) on a Au electrode was conducted using a Ni–NTA-modified Au electrode

Table 1. Summary of vis–NIR spectroscopic data of the LH1 complex, including Zn–BChl *a* as a cofactor bacteriochlorophyll pigment

Entry	Reconstituted complexes (or a native membrane of photosynthetic bacterium)	λ_{max}/nm				
		spx or sphe			Q_x band of Zn–BChl *a*	Q_y band of Zn–BChl *a*
1	LH1-α/LH1-β + Zn–BChl *a*	—	—	—	589	858
2	MBP–rubα-YH/LH1-β + Zn–BChl *a*	—	—	—	590	858
3	LH1-α/LH1-β + Zn–BChl *a* + spx[a]	483	514	548	589	864
4	MBP–rubα-YH/LH1-β + Zn–BChl *a* + spx	482	513	548	590	864
5	LH1-α/LH1-β + Zn–BChl *a* + sphe[a]	442	469	502	589	865
6	MBP–rubα-YH/LH1-β + Zn–BChl *a* + sphe	442	469	502	590	865
7	Chromatophore of *Acp. rubrum*[b]	486	515	549	590	864

[a]Reference 11. [b]This is a purple photosynthetic bacterium containing Zn–BChl *a* as ≈81% of the bacteriochlorophyll cofactor.[12]

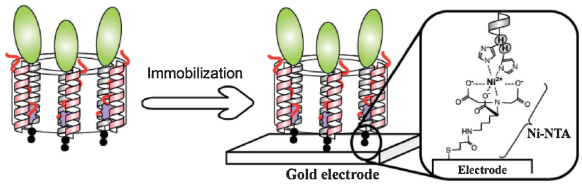

Figure 2. Immobilization of the MBP-modified reconstituted LH1 complex on a Ni–NTA-modified Au electrode.

(Figure 2). Preparation of the Ni–NTA-modified Au electrode was conducted by SAM preparation using C_2-NTA and subsequent Ni^{2+} coordination.[16] A Au electrode (9 mm × 20 mm) was immersed in a solution of the reconstituted complex (approximately 0.2 μM) at 4 °C overnight, after which it was washed several times with 0.78% OG solution and MilliQ water. Figure 3 shows absorption spectra of the reconstituted complex assembled onto the electrode. The Q_y band is clearly consistent with that in the solution, indicating that the complex was successfully immobilized without denaturation. When the immobilized complex was treated with 0.5 M imidazole solution, the Q_y absorption band was completely diminished, indicating that the complex was immobilized via Ni–NTA–His-tag interaction as shown in Figure 2.

To estimate the size of the reconstituted LH1-type complex by AFM, the complex was incorporated into a lipid bilayer (EggPC) via detergent removal from a lipid-detergent comicellar solution and subsequent purification by sucrose density-gradient ultracentrifugation. The resulting proteoliposomal solution was put on a freshly cleaved mica surface. Addition of $MgCl_2$ solution gave planar lipid patches that incorporated the reconstituted MBP–rubα-YH-based LH1-type complex (Figure 4a). The heights of the bilayer patches were 4 nm. On the surface mem-

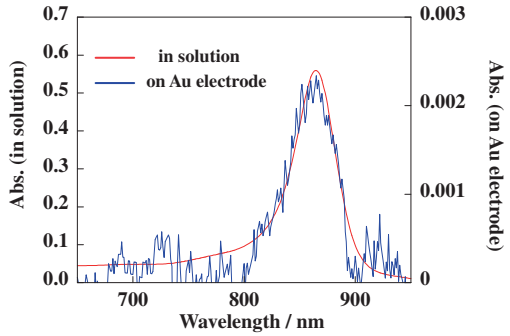

Figure 3. Comparison of the vis–NIR spectra of the MBP-modified LH1 complex (Entry 6) in a 0.78% OG micelle solution (red) and on the Ni–NTA-modified Au electrode (blue).

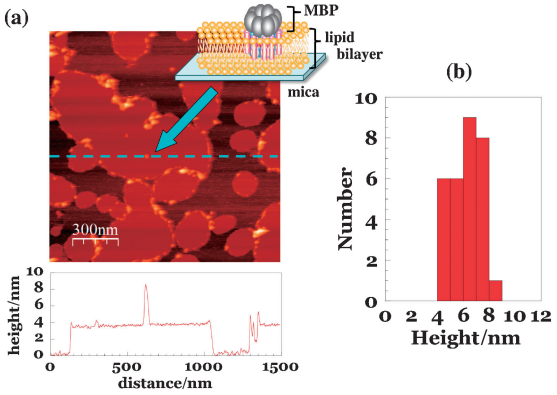

Figure 4. (a) AFM observation of the planar lipid membrane in the presence of the reconstituted LH1-type complexes. (b) A histogram of height of the protrusion on a lipid bylayer.

branes, 4–8 nm protrusions were clearly observed (Figure 4b). X-ray crystallography has revealed that the structure of MBP itself (PDB: 1N3W) is ellipsoidal with dimensions of 30 × 40 × 65 Å;[17] therefore, the observed protrusions can reasonably be assigned to MBP portions on the N-terminal regions of the LH1-type complex as shown in Figure 4. The diameter of the reconstituted MBP–rubα-YH-based LH1-type complex can be calculated to be ≈21 nm on the basis of X-ray crystallographic structures of LH1-RC (PDB: 1PYH) and MBP. The observed diameters (30–50 nm) were larger than the estimated value, and this discrepancy may have resulted from a tip dilatation phenomenon[18] and/or association of the complex in the membrane. In a non-lipid bilayer environment, only featureless complex particles were observed. Thus, the MBP portion acts as a landmark for observation by AFM.

In summary, a bacterial photosynthetic antenna polypeptide (LH1-α) was synthesized as a fusion protein with maltose-binding protein (MBP) and His-tag domains (MBP–rubα-YH) using an *E. coli* expression system. The MBP–rubα-YH was successfully reconstituted with LH1-β polypeptide and pigments (Zn–BChl *a*, carotenoid) to form an LH1-type complex, regardless of the presence of a large hydrophilic MBP portion, which does not sterically hinder the complex formation. The reconstituted complex was immobilized onto a Ni–NTA-modified Au electrode without denaturation. When the complex was incorporated into a planar lipid bilayer, assembled MBP portions were clearly observed by AFM. Taken together, the results of this study suggest that the genetically engineered MBP–rubα-YH is useful for construction of artificial photosynthetic antenna systems based on the promising methodology using functional hydrophilic domains, His-tag and MBP for immobilization onto electrodes with a defined orientation and as a molecular landmark for AFM observation at the molecular level, respectively.

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References and Notes

1 a) S. Karrasch, P. A. Bullough, R. Ghosh, *EMBO J.* **1995**, *14*, 631. b) S. J. Jamieson, P. Wang, P. Qian, J. Y. Kirkland, M. J. Conroy, C. N. Hunter, P. A. Bullough, *EMBO J.* **2002**, *21*, 3927.
2 A. W. Roszak, T. D. Howard, J. Southall, A. T. Gardiner, C. J. Law, N. W. Isaacs, R. J. Cogdell, *Science* **2003**, *302*, 1969.
3 G. McDermott, S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell, N. W. Isaacs, *Nature* **1995**, *374*, 517.
4 J. Koepke, X. Hu, C. Muenke, K. Schulten, H. Michel, *Structure* **1996**, *4*, 581.
5 N. P. Reynolds, S. Janusz, M. Escalante-Marun, J. Timney, R. E. Ducker, J. D. Olsen, C. Otto, V. Subramaniam, G. J. Leggett, C. N. Hunter, *J. Am. Chem. Soc.* **2007**, *129*, 14625.
6 M. Escalante, A. Lenferink, Y. Zhao, N. Tas, J. Huskens, C. N. Hunter, V. Subramaniam, C. Otto, *Nano Lett.* **2010**, *10*, 1450.
7 a) Y. Suemori, M. Nagata, Y. Nakamura, K. Nakagawa, A. Okuda, J. Inagaki, K. Shinohara, M. Ogawa, K. Iida, T. Dewa, K. Yamashita, A. Gardiner, R. J. Cogdell, M. Nango, *Photosynth. Res.* **2006**, *90*, 17. b) Y. Suemori, K. Fujii, M. Ogawa, Y. Nakamura, K. Shinohara, K. Nakagawa, M. Nagata, K. Iida, T. Dewa, K. Yamashita, M. Nango, *Colloids Surf., B* **2007**, *56*, 182. c) M. Kondo, Y. Nakamura, K. Fujii, M. Nagata, Y. Suemori, T. Dewa, K. Iida, A. T. Gardiner, R. J. Cogdell, M. Nango, *Biomacromolecules* **2007**, *8*, 2457.
8 M. Ogawa, R. Kanda, T. Dewa, K. Iida, M. Nango, *Chem. Lett.* **2002**, 466.
9 T. Dewa, T. Yamada, M. Ogawa, M. Sugimoto, T. Mizuno, K. Yoshida, Y. Nakao, M. Kondo, K. Iida, K. Yamashita, T. Tanaka, M. Nango, *Biochemistry* **2005**, *44*, 5129.
10 P. S. Parkes-Loach, J. R. Sprinkle, P. A. Loach, *Biochemistry* **1988**, *27*, 2718.
11 K. Nakagawa, T. Nakano, N. Fukui, A. Nakashima, S. Sakai, T. Mizuno, T. Dewa, K. Iida, A. T. Gardiner, R. J. Cogdell, R. Fujii, H. Hashimoto, M. Nango, *Carotenoid Sci.* **2009**, *14*, 62.
12 N. Wakao, N. Yokoi, N. Isoyama, A. Hiraishi, K. Shimada, M. Kobayashi, H. Kise, M. Iwaki, S. Itoh, S. Takaichi, Y. Sakurai, *Plant Cell Physiol.* **1996**, *37*, 889.
13 C. M. Davis, P. S. Parkes-Loach, C. K. Cook, K. A. Meadows, M. Bandilla, H. Scheer, P. A. Loach, *Biochemistry* **1996**, *35*, 3072.
14 C. M. Davis, P. L. Bustamante, P. A. Loach, *J. Biol. Chem.* **1995**, *270*, 5793.
15 Y. Kakitani, J. Akahane, H. Ishii, H. Sogabe, H. Nagae, Y. Koyama, *Biochemistry* **2007**, *46*, 2181.
16 M. Murata, C. Gouda, K. Yano, S. Kuroki, T. Suzutani, Y. Katayama, *Anal. Sci.* **2003**, *19*, 1355.
17 J. C. Spurlino, G. Y. Lu, F. A. Quiocho, *J. Biol. Chem.* **1991**, *266*, 5202.
18 K. Fukui, S. Sugiyama, Y. Iwasawa, *Phys. Chem. Chem. Phys.* **2001**, *3*, 3871.
19 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

