Construction of Photosynthetic Antenna Complex Using Light-harvesting Polypeptide-α from Photosynthetic Bacteria, *R. rubrum* with Zinc Substituted Bacteriochlorophyll *a*

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The light-harvesting (LH)-α polypeptide isolated from *R. rubrum* only organized zinc bacteriochlorophyll *a* (Zn-BChl *a*) complex in n-octyl-β-D-glucopyranoside (OG) micelle, analogous to the LH1-type complex of photosynthetic bacteria.

The light-harvesting (LH)-α and -β polypeptides of photosynthetic bacteria organize a bacteriochlorophyll *a* (BChl *a*) complex according to cooperative interactions between the LH polypeptides and BChl *a* so that an efficient energy transfer between bacteriochlorophylls may occur.[1–4] It is interesting that the histidine residue in the hydrophobic core of the LH-α and -β polypeptides in the LH complex coordinates with a Mg atom in the BChl *a*, and tryptophan or polar amino acid residue at the C-terminal segment of the LH polypeptides may bind with the C3 acetyl and C13[1] carbonyl groups of BChl *a* by hydrogen-bonding, causing a large red-shift of the Qy absorption band of BChl *a*.[1] It is known that an equimolar mixture of the native LH-α and LH-β polypeptides, separately isolated from photosynthetic bacteria forms a subunit-type complex with Zn-BChl *a* in OG micelle at 25 °C and forms a LH1-type complex on cooling the sample to 4 °C, consistent with BChl *a*.[1] However, the native LH-α or -β polypeptide only does not form the LH 1-type complex with BChl *a* at 4 °C.[1]

In this paper, we first report that LH-α polypeptide, separately isolated from *R. rubrum*, only can assemble the LH1-type complex of photosynthetic bacteria in OG micelle, using Zn-BChl *a*. The key to the assembly is of providing insight into the reasons why the LH-α polypeptide only forms the LH1-type complex with Zn-BChl *a* but do not form the complex with BChl *a*. We selected 1α-helix polypeptides, Cut-α polypeptide and Type 1 (Scheme 1), which have similar amino acid sequence to the hydrophobic core of the native LH-α polypeptide from photosynthetic bacteria, *R. rubrum*. Cut-α polypeptide and Type 1 were synthesized to see the effects of the amino acid sequence at the N-terminal segment of the LH-α polypeptide on forming the LH complex as well as the sequence at the C-terminal segment. Cut-α polypeptide was prepared as described in our previous paper.[4] Type 1 was synthesized by the solid-phase peptide synthesis method on Rink amide resin, using Fmoc-protected amino acids as described previously.[5a] The desired polypeptides were purified by HPLC. These polypeptides gave their expected molecular mass analyzed by TOF-MS (Cut-α: 4854, Type 1: 4030). CD spectra of these polypeptides showed α-helical structures in OG micelle (α-helix content, LH-α: 44.2%, Cut-α: 56.8%, Type 1: 35.2%).[5a] The native LH-α or LH-β polypeptide also was separately isolated from LH1 complex of *R. rubrum*. The molecular assembly of Zn-BChl *a* or BChl *a* by LH and its model polypeptides was carried out according to the reconstitution method, and Zn-BChl *a* or BChl *a* was obtained as described previously.[4]

	N-terminal	← Hydrophobic Core →	C-terminal
LH-α	MWRIWQLFDPRQ	ALVGLATFLFVLALLIHFILLST	ERFNWLEGASTKPVQTS
Cut-α	PRQ	ALVGLATFLFVLALLIHFILLST	ERFNWLEGASTKPVQTS
Type1	CGGDPRQ	ALVGLATFLFVLALLIHFILLST	ERFNWL
LH-β	EVKQESLSGITEGEAKEFHK	IFTSSILVFFGVAAFAHLLVWIW	RPWVPGPNGYS

Scheme 1. Amino acid sequences of the light-harvesting polypeptides from *R. rubrum* and their synthetic model polypeptides.

Table 1 shows the Qy absorption bands and CD signals of Zn-BChl *a* or BChl *a* in the presence of the native LH-α and -β, the LH-α, the LH-β, Cut-α and Type 1. It is known that an equimolar mixture of the LH-α and -β polypeptides from *R. rubrum* with Zn-BChl *a* forms the subunit-type complex absorbing 809 nm in 0.78% OG micellar solution at 25 °C, and forms the LH1-type complex absorbing 858 nm at 4 °C, respectively. This is consistent with the complex using BChl *a* in the presence of the LH-α and -β polypeptides as shown in Table 1.[5]

Table 1. UV-vis. and CD spectral data of Zn-BChl *a* or BChl *a* in the presence of the LH polypeptides[a]

Polypeptides	BChl *a* derivatives	Qy band/nm 25 °C	Qy band/nm 4 °C	CD spectra λ_{max}/nm ($10^{-4}\theta$)	
LH-α	[Zn]-BChl *a*	802	863	866(−20)	837(4.7)
Cut-α		802	863	871(11)	844(−9.6)
Type 1		802	863	872(−24)	845(14)
LH-β		809	830	810(−20)	837(32)
LH-α and -β		809	858	868(7.6)	838(−6.2)
LH-α	BChl *a*	777[d]	777[d]	no signal	
Cut-α[d]		777[d]	871[b,c,d]	882(10)[d]	854(−10)[d]
Type 1		777	874[c]	882(−19)	851(10)
LH-β		818	818	820(−5.5)	
LH-α and -β		818[d]	870[d]	883(7.1)[d]	856(−5.7)[d]

[a]Measured in 0.78% OG solution (phosphate buffer pH 7.0), [Zn-BChl *a* or BChl *a*] = 2.4×10^{-6} mol dm^{-3}, [polypeptide] = 3.4×10^{-6} mol dm^{-3}. [b]The Qy band-shift was not reversible with temperature. [c]Shoulder at 777 nm. [d]See in ref. 4.

Figure 1 shows the Qy absorption bands of Zn-BChl *a* and BChl *a* in the presence of the LH-α polypeptide only in the OG micelle. The Qy band of BChl *a* in the presence of the LH-α polypeptide was observed at 777 nm, corresponding to the band of BChl *a* monomer in acetone (Table 1). Interestingly, the Qy band of Zn-BChl *a* was red-shifted to 802 nm at 25 °C and further red-

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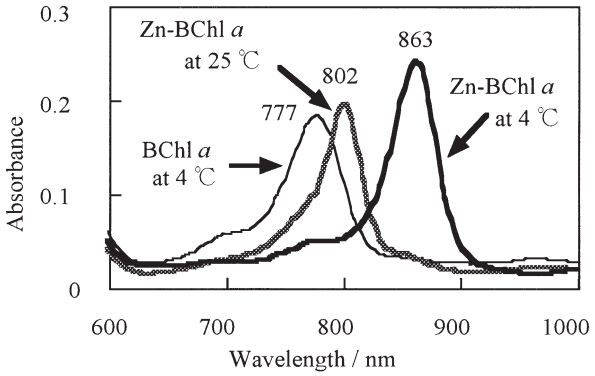

Figure 1. Absorption spectra of Zn-BChl *a* and BChl *a* in the presence of LH-α polypeptide from *R. rubrum* in 0.78% OG solution. Concentrations: polypeptide $= 3.4 \times 10^{-6}$ mol dm^{-3}, Zn-BChl *a* or BChl *a* $= 2.4 \times 10^{-6}$ mol dm^{-3}.

shifted to 863 nm on cooling the sample to 4 °C, analogous to the subunit-type complex absorbing 809 nm at 25 °C and of the LH1-type complex absorbing 858 nm at 4 °C, respectively. These differences in the Qy band between Zn-BChl *a* and BChl *a* in the presence of the LH-α polypeptide only were not observed for the complex formation using the LH-α and -β polypeptides (Table 1). Small-angle X-ray scattering (SAXS) and dynamic light scattering (DLS) measurements revealed that the radius of gyration for the complex between the LH-α polypeptide only with Zn-BChl *a* in the OG micelle was 3.7 nm at 25 °C from the data of SAXS and 28 nm at 4 °C from the data of DLS, respectively, corresponding to that of the native subunit- and LH1-type complexes, respectively.[6] Further, a large split-CD signal around the Qy band of Zn-BChl *a* was observed in the presence of the LH-α polypeptide only at 4 °C (Table 1). This large θ value of CD shows the strong excitonic coupling of the Zn-BChl *a* complex induced by the LH-α polypeptide which is similar to that observed in the presence of the LH-β polypeptide only, implying the strong association of Zn-BChl *a* in comparison to that of the BChl *a*. It is considered that a zinc atom in the porphyrin ring strongly binds with imidazole residue of the histidine in comparison to a Mg atom in the porphyrin ring.[7] These results are consistent with that the binding constant of Zn-BChl *a* complex with the LH-α and -β polypeptides for the subunit–type complex formation is larger by above 100 times than that of BChl *a* complex.[1] Thus, it is considered that the strong association of Zn-BChl *a* with the LH-α polypeptide may affect the LH 1-type complex formation. Resonance Raman spectra indicated that the absorptions of the C3 acetyl and C13^1 carbonyl groups of Zn-BChl *a* were down field-shifted by hydrogen-bonding due to the presence of the LH-α polypeptide in OG micelle, respectively. These UV-vis., CD, SAXS, DLS and Raman spectral data indicate that the LH-α only can form the Zn-BChl *a* complex in the OG micelle, analogous to the subunit-type complex and the LH1-type complex using the LH-α and -β polypeptides, respectively, depending on the temperature.

Alternatively, to see the effects of the amino acid sequence at the N- or C-terminal segment of the LH-α polypeptide on the formation of the LH complex, we examined the molecular assembly of Zn-BChl *a* or BChl *a* using Type 1 and Cut-α, respectively. The Qy band of Zn-BChl *a* in the presence of Type 1 was red-shifted to 802 nm at 25 °C and further red-shifted to 863 nm at 4 °C, which is similar to that seen in the presence of Cut-α or the LH-α polypeptide (Table 1). Further, a large split CD signal at the Qy band of Zn-BChl *a* was observed due to the presence of Type 1, consistent with the signal in the presence of Cut-α or the LH-α polypeptide (Table 1). Interestingly, these red-shifted Qy band of Zn-BChl *a* and the large split CD signal at 4 °C were similar to those seen for BChl *a* (Table 1). Comparing the amino acid sequence at the N-terminal segments of these polypeptides on the LH1-type complex formation with Zn-BChl *a* or BChl *a*, these data indicate that the amino acid residues from M to F at the N-terminal segment of the LH-α polypeptide essentially account for the difference in the complex formation between Zn-BChl *a* and BChl *a*. Thus, it is concluded that amino acid residues at the N-terminal segment cause the difference of the complex formation between Zn-BChl *a* and BChl *a* and no influence of the amino acid sequence at the C-terminal segment on the LH1-type complex formation is observed. Appropriate analogues of the LH-α are useful in constructing an artificial LH complex as well as in providing insight into the effect of polypeptide structure on forming the LH complex of photosynthetic bacteria.

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References and Notes

1 P. S. Parkes-Loach and P. A. Loach, in "Anoxygenic Photosynthetic Bacteria," ed. by R. E. Blankenship, T. M. Madigan, and C. E. Bauer, Kluwer Academic Publishers, Amsterdam (1995), Chap. 21.
2 T. Pullerits and V. Sundstrome, *Acc. Chem. Res.*, **29**, 381 (1996).
3 G. McDermott, S. M. Prince, A. A. Freer, A. M. Hawthornthwaite-Lawless, M. Z. Papiz, R. J. Cogdell, and N. W. Isaacs, *Nature*, **374**, 517 (1995).
4 K. A. Meadows, K. Iida, K. Tsuda, P. A. Recchia, B. A. Heller, B. Antonio, M. Nango, and P. A. Loach, *Biochemistry*, **34**, 1559 (1995).
5 a) A. Kashiwada, N. Nishino, Z.-Y. Wang, T. Nozawa, M. Kobayashi, and M. Nango, *Chem. Lett.*, **1999**, 1301. b) A. Kashiwada, H. Watanabe, T. Mizuno, K. Iida, T. Miyatake, H. Tamiaki, M. Kobayashi, and M. Nango, *Chem. Lett.*, **2000**, 158. c) A. Kashiwada, Y. Takeuchi, H. Watanabe, T. Mizuno, H. Yasue, K. Kitagawa, K. Iida, Z.-Y. Wang, T. Nozawa, H. Kawai, T. Nagamura, Y. Kurono, and M. Nango, *Tetrahedron Lett.*, **41**, 2115 (2000).
6 The data were analyzed by using standard Guinier analysis (A. Guinier and G. Fournet, "Small Angle Scattering," Wiley, New York (1955)), fitting with a form factor of a sphere. The data indicated that the diameter of the complex corresponding to that of the subunit-type complex or LH1-type complex between BChl *a* and the LH-α/-β polypeptides from *R. rubrum* in the OG micelle is 3.8 nm from SAXS measurement at 25 °C or 22 nm from DLS measurement at 4 °C.
7 The axial coordination ability was determined by the intensity of induced CD signals of L-histidine methyl ester (L-HisOMe)-linked metallo-mesoporphyrin monomethyl esters (MPMME) in $CHCl_3$, indicating that the ability was enhanced in the order: ZnMPMME-L-HisOMe > MgMPMME-L-HisOMe > NiMPMME-L-HisOMe.