S1-021

Construction of artificial light-harvesting complex using light-harvesting polypeptide or its model synthetic polypeptides with zinc substituted bacteriochlorophyll *a*

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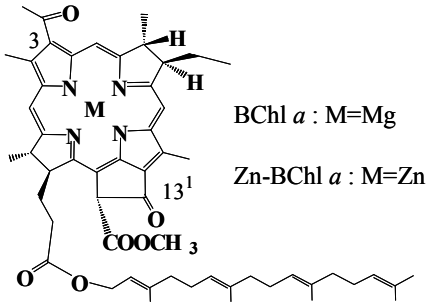
Keywords: artificial light-harvesting complex, self-molecular assembly, zinc bacterio-chlorophyll a, lipid bilayer

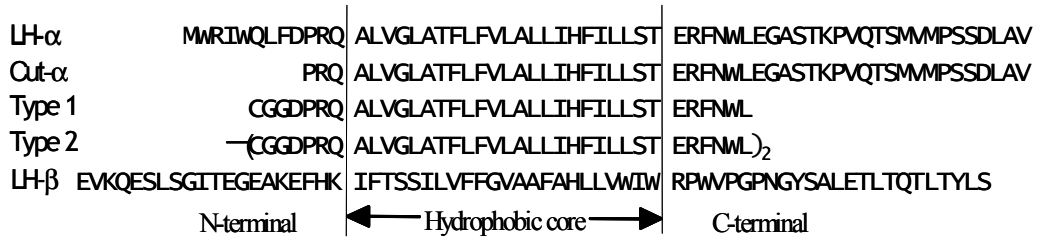
Introduction

The LHα and -β polypeptides of photosynthetic bacteria organize a BChl *a* complex according to cooperative interactions between the LH poly- peptides and BChl *a* so that an efficient energy transfer between bacteriochloro-phylls may occur. It is interesting to note in the LH complex that the histidine residue in the hydrophobic core of the LH polypeptide coordinates with the Mg atom in the BChl *a*, and tryptophane or polar amino acid residue in the C-terminal segment of the LH polypeptides may bind with the C3 acetyl and C13[1] keto carbonyl of BChl *a* hydrogen-bonding, causing a large red-shift of the Qy absorption band of BChl *a* (Parkes-Loach et al., 1995). Work from several laboratories has demonstrated self-assemblies of porphyrins by using synthetic polypeptides to organize artificial hemoprotein models. However, there has been little study of molecular assembly of chlorophylls by using synthetic polypeptides to organize an artificial LH complex.



Scheme 1. Structure of BChl *a* derivatives

In this paper, we examine the molecular assembly of bacteriochlorophylls (Scheme 1) with LH-α polypeptide separartely isolated from *R. rubrum* and its synthetic model polypetides (Scheme 2) in lipid bilayers as well as in OG micelle. The key to the molecular assembly is to form an artificial LH complex using Zn-BChl *a* in lipid bilayers. We reason that Zn-BChl *a* is used because of its stability in comparison to BChl *a*. We selected LH-α model polypeptides, Cut-α polypeptide (Meadows et al., 1995) and synthetic LH-α model peptides, Types 1 and 2 which have similaramino acid sequence to the hydrophobic core



Scheme 2. Amino acid sequences of LH-α, -β polypeptides of *R.rubrum* and synthetic LH-α model polypeptides.

of the native LH-α polypeptide from*R. rubrum* to see the effects of the amino acid sequence in the N-terminal segment of the LH-α polypeptide on forming the LH complex.

Materials and methods

The native LH-α and -β polypeptides were separately isolated from LH1 complex of *Rhodospirillum rubrum* (*R. rubrum*) and Zn-BChl *a* or BChl *a* was obtained as described previously (Meadows et al., 1995; Parkes-Loach et al., 1995). Cut-α polypeptide was prepared as described in our previous paper (Meadows et al., 1995). Model polypeptides were synthesized by the Fmoc method using peptide synthesizer. The desired polypeptides were purified by Sephadex G-75 column and then by HPLC. The HPLC analysis confirmed their purity was high enough (>95%) to assemble these polypeptides. These polypeptides were analyzed by TOF-MS to give the expected molecular mass. The molecular assembly of Zn-BChl *a* or BChl *a* by synthetic polypeptides was carried out according to the reconstitution method using the native LH-α and -β polypeptides (Meadows et al., 1995). It is known that an equimolar mixture of the LH-α and -β polypeptides with Zn-BChl *a* or BChl *a* forms the subunit-type complex absorbing 809 or 818 nm, respectively in 0.78% OG at 25 °C and then forms the LH1-type complex absorbing 858 or 870 nm, respectively, at 4 °C as shown in Table 1 (Meadows et al., 1995). The SAXS data were analyzed by using standard Guinier analysis, fitting with a form factor of a sphere.

Results and discussion

Table 1 shows the Qy absorption bands and CD signal of Zn-BChl *a* in the presence of the native LH polypeptides and its synthetic model polypeptides. Figure 1 shows the Qy absorption bands of Zn-BChl *a* and BChl *a* in the presence of the LH-"a" polypeptide in 0.78% OG solution. The Qy band of BChl *a* in the presence of the LH-α polypeptide was observed at 777 nm, corresponding to the band of Zn-BChl *a*monomer at 774 nm. Interestingly, the Qy band of Zn-BChl *a* was red-shifted to 802 nm at 25 °C and further red-shifted to 863 nm on cooling to 4 °C, analogous to the complex-forming for the subunit- to the complex-forming for the subunit-type absorbing 809 nm at 25 °C and for the LH1-type complex absorbing 858 nm at 4 °C, respectively (Parkes-Loach et al., 1995). These

differences in the Qy band between Zn-BChl *a* and BChl *a* in the presence of the LH-α polypeptide were not observed for the complex-forming using LH-α and -β polypeptides or LH-β polypeptide only (Table 1). Small- angle X-ray scattering (SAXS) and dynamic light scattering (DLS) measurements revealed that the radius of gyration

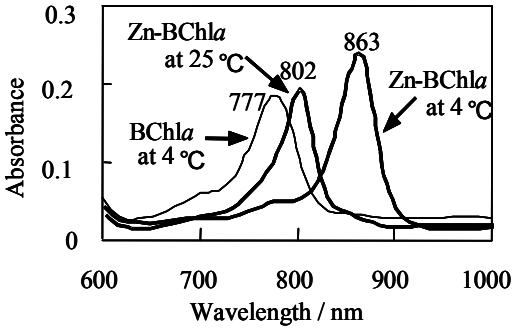


Figure 1. UV-vis. spectra of Zn-BChl *a* and BChl *a* in the presence of LH-α polypeptide from *R. rubrum* in 0.78 % OG solution.

for the complex between the LH-α poly- peptide with Zn-BChl *a* in 0.78 % OG was 3.7 nm at 25 °C from the data of SAXS and 28 nm at 4 °C from the data of DLS, corresponding to that of the subunit- and the LH1-type complex, respectively. Furthermore, a large split-CD

Table 1. UV-vis. and CD Spectral Data of Zn-BChl*a* in the Presence of the LH Polypeptide and Its Model Polypeptides [a]

	UV-vis. spectra Qy band / nm		CD spectra at 4 ℃ θ (10^{-4} deg cm^{-2} $dmol^{-1}$)	
Polypeptides	25 ℃	4 ℃		
LH-α	802	863	866(-20)	837(4.7)
Cut-α	802	863	871(10)	844(-11)
Type 1	802	863	872(-24)	846(14)
Type 2	802	863	872(-24)	846(14)
LH-α and LH-β	809	858	868(7.6)	838(-6.2)

[a] Measured in 0.78 % OG solution (phosphate buffer pH 7.0), [Zn-BChl*a*] = 2.4 × 10^{-6} mol dm^{-3}, [polypeptide] = 3.4× 10^{-6} mol dm^{-3}.

signal around the Qy band of Zn-BChl *a* was observed in the presenceof the LH-α polypeptide at 4 °C (Table 1). This large "q" value of CDimplies that strong association of the Zn-BChl *a* complex induced by the LH-α polypeptide. These UV-vis., CD, SAXS, and DLS data indicate that the LH-α polypeptide selectively organizes a Zn-BChl *a* complex in OG solution, analogous to the subunit-type complex and the LH1-type complex, respectively, depending on the temperature.

Alternatively, to see the effects of amino acid sequence in the N-terminal segment of the LH-α polypeptide on the LH complex-forming, we examined the molecular assembly of Zn-BChl *a* or BChl *a* with Types 1 and 2, 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, consistent with the Qy bands in the presence of the LH-α polypeptide at 25 and 4 °C (Figure 1 and Table 1). Furthermore, 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 the LH-α polypeptide (Table 1). Interestingly, this red-shift and a large split CD signal of the Qy band at the Qy band were observed for BChl *a* at 4 °C as well as for Zn-BChl *a* (Table 1). Comparing the amino acid sequence in the N-terminal segments of these polypeptide on the LH1 complex-forming with Zn-BChl *a* or BChl *a*, these data indicate that the amino acid residues from M to F in the N-terminal segment of the LH-α polypeptide essentially account for the difference in the complex-forming between Zn-BChl *a* and BChl *a*. Thus, it is considered that cationic amino acid residues from M to F in the N-terminal segment ca use the difference of the complex-forming between Zn-BChl *a* and BChl *a*. The strong association of Zn-BChl *a* induced by the LH-α polypeptide is likely to overcome the repulsion between the cationic amino acid residues and thus cause the LH 1-type complex-forming. Interestingly, no difference in the red-shift and the split-CD signal at the Qy band of Zn-BChl *a* in the presence of Type 2 and Type 1 was observed at 4 °C (Table 1), indicating no influence of the disulfide-linkage at the N-terminal segment on the complex-forming.

To examine further the molecular assembly of Zn-BChl *a* with the LH-α polypeptide only, the complex-forming between Zn-BChl *a* and the LH-α polypeptide in lipid bilayers was carried out as follows. A solution of liposomal membrane containing PE, PG, CL and the LH-α (2:1:1) was mixed with a solution of liposomal membrane containing PE, PG, CL and Zn-BChl *a* (2:1:1). Then, OG was added to the mixed solution until 0.78 % OG solution was prepared, and OG was removed by dialysis for two day to fuse these liposomal membranes. The proof of the complex-forming in the mixed lipid bilayers was noted by monitoring the Qy absorption band-shift of Zn-BChl *a*. For example, after the dialysis, the Qy band in the mixed lipid bilayers was red-shifted from 777 to 863 nm due to the presence of the LH-α at 4 °C, analogous to the LH1-type complex in the OG micelle. However, the red-shift of the Qy band was not observed in the absence of the LH-α. This is the first report that the LH polypeptide only forms an artificial LH complex with Zn-BChl *a* in lipid bilayers as well as in the OG micelle. Appropriate analogues of the LH-α and its model synthetic polypeptides are useful in constructing an artificial LH complex of photosynthetic bacteria as well as in providing insight into the effect of polypeptide structure on forming the complex.

Acknowledgments

M.N. thanks Dr. P. Parkes-Loach and Prof. P. A. Loach, Northwestern University, U.S.A. for a kind gift of photosynthetic bacteria, *R. rubrum*. The present work was partially supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan and Terumo Co. Japan.

References

Meadows, K. A.; Iida, K.; Tsuda, K.; Recchia, P. A.; Heller, B. A.; Antonio, B.; Nango, M.; Loach, P. A. (1995), *Biochemistry* **34**, 1559-1574.

Parkes-Loach, P.S.; Loach, P.A. (1995), Vol.2, Chapt. 21 in *Anoxygenic Photosynthetic Bacteria*, Blankenship, R.E.; Madigan,T.M.; Bauer,C.E.; Eds.; Kluwer Academic Publishers, Amsterdam.