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Molecular assembly of covalently-linked mesoporphyrin dimers with light-harvesting polypeptides

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Abstract

Light-harvesting (LH)-α and -β polypeptides separately isolated from photosynthetic bacteria, *R. rubrum*, organized mesoporphyrin dimers (Scheme 1) in the *n*-octyl-β-D-glucopyranoside (OG) micelle, depending upon the porphyrin structure and temperature. An efficient energy transfer from Zn porphyrin to Ni porphyrin in **7** due to the presence of the LH polypeptides was observed. © 2000 Elsevier Science Ltd. All rights reserved.

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Synthetic porphyrin model compounds can be very helpful in providing insight into the pigment molecular structure of porphyrin-based energy-transfer and electron-transfer processes in photosynthesis.[1,2] Especially, porphyrins play an important role in the energy-transfer process in light-harvesting (LH) polypeptide complex of photosynthesis, where porphyrins such as bacteriochlorophylls (BChls) are assembled according to cooperative interactions between the LH polypeptides and BChls.[3–6] It is interesting to note that the LH polypeptides organize the porphyrin complex so that an efficient energy transfer between porphyrins may occur. However, there has been little study of an artificial energy-transfer system involving LH polypeptides.

In this paper, we examine the molecular assembly of mesoporphyrin dimers **1**–**7** (Scheme 1) using LH-α and -β polypeptides (Scheme 2) from purple photosynthetic bacteria, *R. rubrum* in OG micelle. The key to the molecular assembly is organization of porphyrin dimers using the LH polypeptides to construct an artificial energy-transfer process between porphyrins. The histidine or polar amino acid residue of the LH polypeptides was expected to coordinate the porphyrin dimer,[7] where the Zn or Ni

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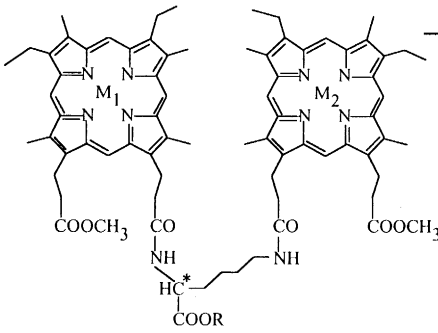
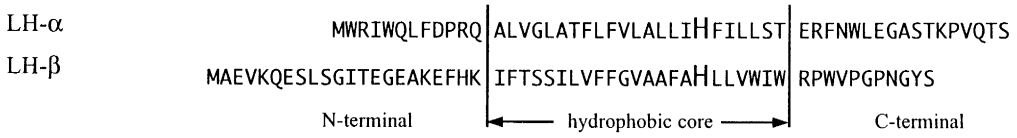
atom and the carboxyl group on the porphyrin might correspond to the Mg atom and C3 acetylcarbonyl group in BChl *a*, respectively.[5] *R. rubrum* was selected because the LH complex had only an LH1 complex. LH-α and -β polypeptides from *R. rubrum* were extracted by $CHCl_3$/MeOH and purified by Sephadex LH-60 gel chromatography and HPLC.[6] Mesoporphyrin dimers **1**–**7** (Scheme 1) were prepared as follows. Mesoporphyrin monomethyl ester, H_2MPMME was prepared by reduction of protoporphyrin, and then by acid-hydrolysis. Its Zn or Ni complex, ZnMPMME or NiMPMME was prepared as described previously.[2,8] To introduce the lysine residue, H_2MPMME or ZnMPMME was treated with *N*-hydroxysuccinimide (HONSu)/DCC and Boc-L-lysine or D-isomer, and the product was further treated with H_2MPMME or ZnMPMME or NiMPMME and HONSu/DCC. The products **1**–**4** were purified by column chromatography on silica gel. Compounds **5** and **6** were prepared by esterification of **1** or **3**, respectively, with $SOCl_2$/MeOH in $CHCl_3$. ^{1}H NMR and FAB mass spectra support the assigned structure of the porphyrin derivatives.[9] Molecular assembly of the porphyrin derivatives with the LH polypeptides was performed in the OG micelle as described in the previous paper.[6]



		M_1	M_2	R
1	MP-L-Lys(MP)	H_2	H_2	OH
2	ZnMP-L-Lys(MP)	Zn	H_2	OH
3	ZnMP-L-Lys(ZnMP)	Zn	Zn	OH
4	ZnMP-D-Lys(ZnMP)	Zn	Zn	OH
5	MP-L-Lys(OMe)(MP)	H_2	H_2	OCH_3
6	ZnMP-L-Lys(OMe)(ZnMP)	Zn	Zn	OCH_3
7	ZnMP-L-Lys(NiMP)	Zn	Ni	OH

Scheme 1. Structures of mesoporphyrin dimers



Scheme 2. Amino acid sequences of the light-harvesting polypeptides from *R. rubrum*

The Soret bands of UV–vis absorption and CD signals for mesoporphyrin dimers **1**–**7** in the presence of the LH-α and -β polypeptides are summarized in Table 1. Fig. 1 shows UV–vis absorption spectra of **3** in the presence and absence of the LH polypeptides at 25 and 4°C. The Soret band of **3** was split at 386 and 416 nm, respectively, in presence of the LH polypeptides at 25°C. Interestingly, when cooling the solution to 4°C, the Soret band at 386 nm almost disappeared and, in contrast, the band at 416 nm was largely increased with sharpening. These changes in the Soret band were reversible with temperature, while the Soret band of **3** kept broadening in the absence of the LH polypeptides at 4°C. This red-shift of the Soret band was not observed for **1** (Table 1). These results implied that the axial-coordination of the Zn atom in the porphyrin dimer with the histidine residue in the hydrophobic core of the LH polypeptides caused the red-shift especially at low temperature, consistent with histidine-linked Zn mesoporphyrin, ZnMPMME-L-HisOMe in $CHCl_3$.[10] Similar red-shifts of the Soret band due to the presence of the LH polypeptides were observed for **2**, D-isomer **4**, **6**, **7**, and ZnMPMME, where the band of 386 nm for **2**, **6** or 392 nm for **7** did not disappear (Table 1). A large split-CD signal at the red-shifted Soret band for **3** was observed due to the presence of the LH polypeptides especially when cooling from 25 to 4°C as shown in Fig. 2, implying that the edge-to-edge association of porphyrins induced by the LH polypeptides occurs.

A similar split-CD signal was observed for D-isomer **4**, **2** or **7** but no split-CD signal was observed for its free base derivative **1** and it dimethyl esters **6** (Table 1). These results revealed that the carboxyl group as well as the Zn or Ni atom in the mesoporphyrin dimers is essential for the molecular assembly of the dimers with the LH polypeptides, where the carboxyl group on the dimer may bind with polar amino acid residues in the LH-α or -β polypeptides through hydrogen bonding.[5] Interestingly, no difference in the molecular assembly between D- and L-isomer of the dimer in the presence of the LH polypeptides was observed.

Table 1
UV–vis and CD spectral data of mesoporphyrin dimers in the presence and absence of LH polypeptides

		Soret band / nm		CD	
Dimer[a]	Polypeptides[b]	25 ℃	4 ℃	λ_{max} / nm (10^{-4} θ)	
1	LH-α + LH-β	386	395	402(16)	386(-18)
2	LH-α + LH-β	386 414	386 414	418(-8.2)	412(1.9)
3	LH-α + LH-β	386 416	416	418(-16)	410(9.7)
3	none	386	396	401(19)	384(-24)
4	LH-α + LH-β	386 416	416	418(-14)	411(7.1)
6	LH-α + LH-β	386 410	386 410	406(15)	384(-17)
7	LH-α + LH-β	386 412	392 416	417(-4.5)	410(2.2)
ZnMPMME	LH-α + LH-β	406	416	418(-38)	412(18)

[a][mesoporphyrin] = 3.45×10^{-6} mol dm^{-3} in 0.78% OG solution (phosphate buffer pH 7.0). [b][LH polypeptides] = 3.45×10^{-6} mol dm^{-3} in 0.78% OG solution.

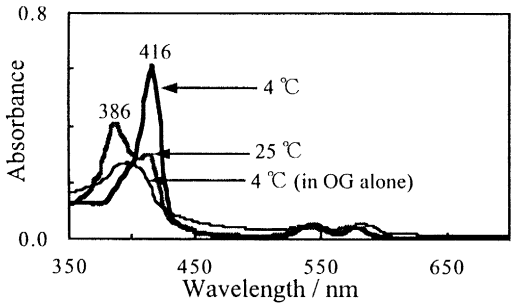


Fig. 1. UV–vis absorption spectra of **3** in the presence and absence of LH-α and -β polypeptides in 0.78% OG solution at 25°C and 4°C. [polypeptide]=3.45 mol dm^{-3}, [**3**]=1.73 mol dm^{-3}

To analyze the molecular dimension of the complex between **3** and the LH-α and -β polypeptides in the OG micelle, the small-angle X-ray scattering (SAXS) was measured.[11] The data indicated that the diameter of the complex between **3** and the LH polypeptides in the OG micelle was 6.6 nm at 4°C, corresponding to that of the subunit-type complex (5.5 nm) between BChl *a* and the LH polypeptide in the OG micelle. Thus, the size of the complex between **3** and the LH polypeptides is comparable to that of the subunit-type complex, which is considered as the complex between the BChl *a* dimer and a pair of LH-α and -β polypeptides, rather than the LH1 type complex.[3]

Alternatively, the fluorescence spectra of mesoporphyrin dimers in the presence of LH-α and -β polypeptides were measured when excited at the wavelength of the Soret band. The largest fluorescence quenching was observed for **7**, where the quenching occurred due to energy-transfer or electron-transfer

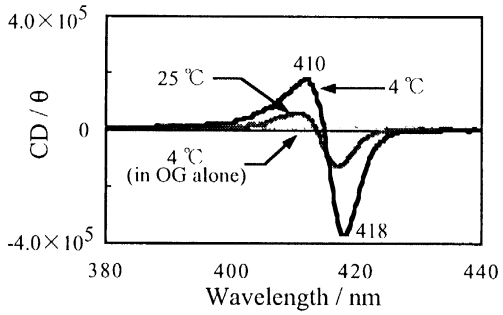


Fig. 2. CD spectra of **3** in the presence and absence of LH-α and -β polypeptides in 0.78% OG solution at 25°C and 4°C. [polypeptide]=3.45 mol dm^{-3}, [**3**]=1.73 mol dm^{-3}

processes between porphyrins associated with LH polypeptides.[12] The most probable mechanism for the observed quenching is likely due to energy transfer by the Förster dipole–dipole interaction. Thus, a value for the quantum efficiency of the relative $I_{H|A}$ value was calculated using the equation written in the caption of Table 2.[2,12–14] As is apparent from Table 2, the most efficient energy-transfer is observed for **7** assembled by the LH-α and -β polypeptides (relative $I_{H|A}$=4.87). This result reveals that the LH polypeptides induce an efficient intramolecular energy-transfer from Zn porphyrin to Ni porphyrin in the hybrid dimer.

Table 2
The quantum efficiencies of the energy transfer

Dimer[a]	Polypeptides[b]	rel $I_{H|A}$[c]
2	LH-α + LH-β	0.472
7	LH-α + LH-β	4.87
ZnMPMME / NiMPMME	LH-α + LH-β	2.28
7	none	1.01

[a][mesoporphyrin] = 3.45×10^{-6} mol dm^{-3} in 0.78% OG solution (phosphate buffer pH 7.0). [b][LH polypeptides] = 3.45×10^{-6} mol dm^{-3} in 0.78% OG solution. [c]rel $I_{H|A} = I_{H|A} / I_{0|A}$. $I_{H|A}$ is the fluorescence intensity of **2** and **7** at 625.0 and 690.5 nm, respectively. $I_{0|A}$ is that of free base and Ni mesoporphyrin monomers at 625.0 and 690.5 nm, respectively.

In conclusion, it is demonstrated that the carboxyl group as well as the Zn or Ni atom in the mesoporphyrin dimers is essential for the molecular assembly of the dimers with the LH polypeptides. Thus, appropriate analogues of porphyrin dimers are useful in constructing efficient energy-transfer processes using LH polypeptides.[15]

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9. Mass spectra: FAB, *m/z*; 1271 for H_2MP-L-Lys-H_2MP **1**; 1335 for ZnMP-L-Lys-H_2MP **2**; 1398 for ZnMP-L-Lys-ZnMP **3**; 1286 for H_2MP-L-Lys(OMe)-H_2MP **5**; 1412 for ZnMP-L-Lys(OMe)-ZnMP **6**. Their stereoisomers were not separated because similar UV–vis and CD spectra of the D-isomer of **3** was observed with **3** due to the presence of the LH polypeptides in the OG micelle (data not shown). More detailed synthetic and analytical results will be reported elsewhere.
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14. The excitation energy was not always transferred to the acceptor due to the quenching caused by the neighboring water, so that the relative emission intensity of acceptor (relative $I_{H|A}$, equation was described in the caption of Table 2) was considered in evaluating the ability of the energy transfer. If Φ_{et} value is close to 1 and the relative $I_{H|A}$ value is increased, an efficient energy transfer is brought in the system.
15. The fluorescence lifetime was measured by YAG laser (532 nm) flash photolysis of ps, in which ZnMPMME was 2 ns (100%) and **3** was composed of 340 ps (40%) and 2 ns (60%), respectively at 4°C. The lifetime of ZnMPMME or **3** in DMSO alone was the same as 2 ns.