Structural Requirements of Zinc Porphyrin Derivatives on the Complex-Forming with Light-Harvesting Polypeptides

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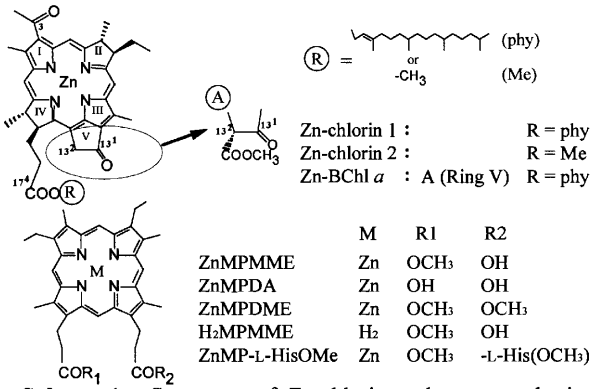
Zn-bacteriochlorins or Zn-mesoporphyrins were successfully assembled with light-harvesting (LH)-α and -β polypeptides separately isolated from photosynthetic bacteria in n-octyl-β-D-glucopyranoside (OG) micelle, depending on the structure of Zn-porphyrin derivatives and on temperature.

It is interesting to note that the light-harvesting (LH) polypeptides of photosynthetic bacteria organize bacteriochlorophyll *a* (BChl *a*) according to cooperative interactions between the LH polypeptides and BChl *a* : the Mg atom in BChl *a* is likely to coordinate with the histidine residue in the hydrophobic core of the LH polypeptides while C3 acetyl and $C13^1$ keto carbonyl of BChl *a* may bind with tryptophane or other polar amino acid residues in the C-terminal of LH polypeptides through hydrogen-bondings.[1] Recently, the presence of Zn bacteriochlorophyll *a* (Zn-BChl *a*) as well as BChl *a* in the LH complex of acidophilic bacterium, *Acidphillum rubrum* was discovered.[2] However, there is little information on the LH complex-forming using Zn-BChl *a*. In this paper, we demonstrate the molecular assembly of Zn-bacteriochlorins or Zn-mesoporphyrins (Scheme 1) with LH-α and -β polypeptides from *Rhodospirillum rubrum* in OG micelle. The key to the molecular assembly is to provide insight into structural requirements of Zn-BChl *a* on the LH complex-forming in photosynthetic bacteria as well as to construct an artificial energy-transfer process using Zn-porphyrins.



Scheme 1. Structures of Zn-chlorin and mesoporphyrin derivatives.

Zn-BChl *a* was extracted and then purified as described in the previous paper.[2] Zn-chlorins 1 and 2 have C3 acetyl and $C13^1$ keto groups as in Zn-BChl *a* but have no $C13^2$ carbomethoxy group. Zn-chlorin 2 was modified esterifying alcohols at position $C17^4$ from phytyl group to methyl group.[3,4]

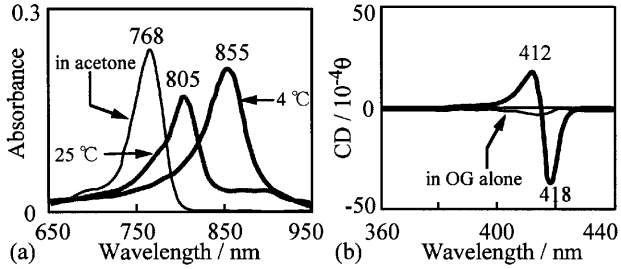


Figure 1. (a)Absorption spectra of Zn-chlorin 1 in the presence and absence of LH-α and -β polypeptides from *R. rubrum* in 0.78% OG solution. Concentrations : polypeptide = 3.45 μM, Zn-chlorin 1 = 2.45μM. (b) CD spectra of ZnMPMME in the presence and absence of LH polypeptides in 0.78% OG solution at 4 ℃. Concentrations : polypeptide = 3.45 μM, ZnMPMME = 3.45 μM.

Synthetic chlorins were purified by column chromatography and then were recrystallized. All mesoporphyrin derivatives were prepared as described in the previous paper.[5] ^{1}HNMR, UV-vis. and mass spectra support the assigned structure of these Zn-porphyrins.[6] LH-α and -β polypeptides were extracted from *R. rubrum* by $CHCl_3$ / MeOH and purified by Sephadex LH-60 gel chromatography and then by HPLC.[7] The LH complex-forming using Zn-porphyrin derivatives in OG micelle was made as previously described.[7-9] It is known that the LH polypeptides form a subunit-type complex with Zn-BChl*a* absorbing at 809 nm in 0.78% OG at 25 °C and form a LH1-type complex absorbing at 858 nm when cooling the complex to 4 °C.[9,10]

Figure 1(a) shows the absorption spectra of the Qy band for Zn-chlorin 1 in the presence of LH-α and -β polypeptides in OG micelle. The Qy band was red-shifted from 768 to 805 nm due to the presence of the LH-polypeptides and further red-shifted to 855 nm when cooling the sample from 25 to 4 °C, where the Qy band of Zn-chlorin 1-monomer in acetone was observed at 768 nm. No red-shift of the Qy band was observed for bacteriopheophytin in the presence of the LH polypeptides. A split CD signal around the Qy band of Zn-chlorin 1 was observed due to the presence of the LH polypeptides at 4 °C (Table 1). These UV-vis. and CD behaviors of Zn-chlorin 1 reveal the LH complex-forming using Zn-chlorin 1, analogous to the LH1-type complex-forming using Zn-BChl *a*. This result indicates that the $C13^2$ carbomethoxy group in Zn-BChl *a* is not essential for forming the LH1-type complex although the group in BChl *a* is necessary on the LH1-type complex-forming using BChl *a*.[10] The difference of contribution of $C13^2$ carbomethoxy group to forming the LH1-type complex between

Table 1. UV-vis. and CD Spectral Data of Zn-chlorin and Mesoporphyrin Derivatives in the Presence and Absence of LH Polypeptides[a]

	Qy or Soret band[b]/ nm			CD
Compound	25 ℃	4 ℃	monomer[c]	λ_{max}/nm (10^{-4} θ)
Zn-chlorin 1	805	855	768	868(7.6) 838(-6.2)
Zn-chlorin 2	770	772 852	765	——[d]
Zn-BChl *a*	809	858	774	867(7.5) 835(-6.5)
ZnMPMME	406	416	402	418(-38) 412(18)
ZnMPMME[e]	402	402		404(-4.8)
ZnMPDA	406	416	402	418(-28) 410(12)
ZnMPDME	406	390 414	402	no signal
H_2MPMME	403	403	400	no signal

[a][Zn-chlorin] = 2.42×10^{-6} mol dm^{-3} , [mesoporphyrin] = 3.45×10^{-6} mol dm^{-3} and [LH polypeptides] = 3.45×10^{-6} mol dm^{-3} in 0.78% OG solution. [b]The Qy band for Zn-chlorin and Zn-BChl *a* and the Soret band for mesoporphyrin. [c]Zn-chlorin in acetone and mesoporphyrin in chloroform (in the absence of LH polypeptides) at 4 ℃. [d]Not measured. [e]In the absence of LH polypeptides.

BChl *a* and Zn-BChl *a* is likely due to the difference of the coordination-ability between the central metal and the histidine residue in the hydrophobic core of the LH polypeptides. Furthermore, we examined the LH complex-forming using Zn-chlorin 2 in OG micelle. The visible absorption spectra indicated that the red-shift of the Qy absorption band, analogous to that for the LH1-type complex using Zn-BChl *a* was observed due the presence of the LH polypeptides, where the Qy band of Zn-chlorin 2-monomer was partially remained (Table 1). These data reveal that the LH complex-forming using Zn-chlorin 2 is less stable in comparison to the complex-forming using Zn-chlorin 1. Thus, the long tailed esterifying alcohols such as the phytyl group at position C17[4] on Zn-BChl *a* is essential for forming a stable LH1-type complex.

Alternatively, to examine further the structural requirements of Zn-BChl *a* on the LH1-type complex-forming, we demonstrated the molecular assembly of Zn-mesoporphyrins with the LH polypeptides. As shown in Table 1, the Soret band of ZnMPMME was observed at 406 nm due to the presence of the LH polypeptides in 0.78% OG solution at 25 °C and further red-shifted to 416 nm when cooling the sample to 4 °C, where the band was observed at 402 nm in the absence of the LH polypeptides. No red-shift of the Soret band was observed for the free-base mesoporphyrin (H_2MPMME) in the presence of LH polypeptides (Table 1). These results revealed that the axial-coordination of the histidine residue in the hydrophobic core of the LH polypeptides with the Zn atom in the porphyrin caused the red-shift of the Soret band, consistent with that of hisidine-linked zinc mesoporphyrin, ZnMPMME-L-HisOMe in $CHCl_3$ (Table 1). Interestingly, the CD spectrum of ZnMPMME showed a large-split CD signal due to the presence of the LH polypeptides especially at 4 °C (Figure 1(b)). This result implies the association of ZnMPMME induced by the LH polypeptides (Table 1). These UV-vis. and CD data suggest that the Zn atom in ZnMPMME coordinates with the histidine residue in the hydrophobic core of the LH polypeptides and then ZnMPMMEs become associated due to the presence of the LH polypeptides especially at low temperature. Interestingly, UV-vis. and CD data for ZnMPMME were similar to those for ZnMPDA having two carboxyl groups but not to those for ZnMPDME having no carboxyl group (Table 1). This result implies that the carboxyl group on the porphyrin ring is essential for the LH-complex-forming using Zn-mesopophyrins, contributed by electrostatic or hydrogen-bonding interaction between the porphyrin and polar amino acid residue of LH polypeptides.[1] Small angle X-ray (SAXS) measurement indicated that the molecular dimension of the LH complex-forming using ZnMPMME in OG micelle at 4 °C was 5.5 nm comparable to that of the LH complex-forming using Zn-BChl *a* at 25 °C (the subunit-type complex).[11] Interestingly, a large fluorescence quenching of ZnMPMME was observed due to the presence of the LH polypeptides in OG micelle. This quenching is likely due to energy transfer or electron transfer between porphyrins associated by the LH polypeptides.[12] These UV-vis., CD, SAXS and fluorescence spectral data reveal that the structural requirements of Zn-porphyrin derivatives on the LH complex-forming with LH polypeptides are as follows: (1) Zn as porphyrin central metal, (2) the carboxyl group on the mesoporphyrin ring, and (3) C3 acetyl carbonyl group and the long tailed esterifying alcohols on the porphyrin ring, where (3) is not essential for the LH compex-forming analogous to the subuit-type complex.

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11 Small angle X-ray scattering and dynamic light scattering measurements indicated that the diameter of the subunit- or LH1-type complex with Zn-BChl *a* was 3.7 nm or 28.0 nm, respectively.
12 Fluorescence spectra of ZnMPMME in the presence of the LH-α and -β polypeptides in OG micelle were measured when excited at the wavelength of the Soret band. The fluorescence lifetime was measured by YAG laser (532nm) flash photolysis of pico sec. The life time for ZnMPMME in the presence of the LH-polypeptides was 2 ns, corresponding to that in chloroform but a fast decay curve was partially observed in comparison to that in chloroform.