Molecular Assembly of Zinc Bachteriochlorophyll *a* by Synthetic Hydrophobic 1α-Helix Polypeptides

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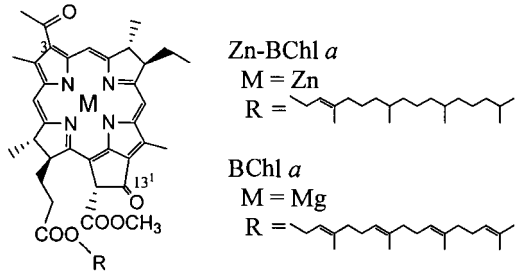
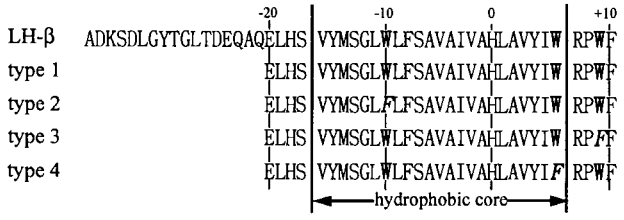
Synthetic 1α-helix hydrophobic polypeptides organized zinc bachteriochlorophyll *a* (Zn-BChl *a*) complex in n-octyl-β-D-glucopyranoside (OG) micelle analogous to the light-harvesting (LH) polypeptide complex of photosynthetic bacteria.

It is interesting to note that the light-harvesting (LH) polypeptides from photosynthetic bacteria organize bachteriochlorophyll *a* (BChl *a*) complex according to cooperative interactions between the polypeptide and BChl *a* so that efficient energy-transfer processes of porphyrins in the LH polypeptides may occur.[1,2] Work from several laboratories has demonstrated self-assemblies of porphyrins by using synthetic polypeptides to organize an artificial hemoprotein model.[3-9] However, there has been little study of the molecular assembly of BChl *a* and its analogues by using synthetic polypeptides to organize an artificial LH model complex.[10]



Scheme 1. Structures of bacteriochlorophyll *a* derivatives.

In this paper, we examine the molecular assembly of Zn-BChl*a* complex (Scheme 1) by using synthetic hydrophobic 1a-helix polypeptides, types 1-4 (Scheme 2) in n-octyl-β-D-glucopyranoside (OG) micelle to organize an artificial LH complex as well as to provide an insight into the effect of amino acid residues of the LH polypeptide on formation of the LH complex. We selecte types 1-4 which were similar sequences to the hydrophobic core of the native LH-β polypeptide from photosynthetic bacteria, *Rhodobacter spheroides* because it is known that the native polypeptide forms a stable subunit-complex, B820 of the LH complex in OG micelle as observed with BChl*a*.[1] Types 1-4 are synthesized to see the effect of tryptophane residue in the amino acid segments on formation of the LH complex as well as to see the N-terminal segment of the native LH-β.[10] 1α-Helix polypeptides were synthesized by the solid-phase peptide synthesis method on Rink amide resin, using Fmoc protected amino acids on an Applied Biosystems peptide synthesizer, model 433A. The desired polypeptides were purified by HPLC.[11,12] These polypeptides gave their expected molecular mass analyzed by TOF-MS (type 1: 3701.2 Da, type 2: 3662.9 Da, type 3: 3662.5 Da, type 4: 3664.2 Da). CD spectra of these polypeptides showed α-helical structures in OG micelle (α-helix content, type 1: 29%, type 2: 32%, type 3: 27%, type 4: 26%, LH-β: 50%). Zn-BChl *a* was used because of its chemical stability in comparison to BChl *a*. Zn-BChl *a* and BChl *a* were obtained as described previously.[13] The molecular assembly of Zn-BChl *a* by synthetic polypeptides was carried out as followed. For example, each 10 nmol of the polypeptides was dissolved in 4.5% OG (50 mM phosphate buffer, pH 7.5) and diluted to 0.90% OG by buffer, and then 7 nmol of Zn-BChl *a* or BChl *a* in acetone was added. The solution was further diluted to 0.78% OG (subunit-type complex-forming condition) and was chilled at 4 °C overnight (LH1-type complex-forming condition),[11,12] in which the concentration of OG (26.7 mM) was around its cmc.[14] It is considered that an equimolar mixture of the native LH-α and LH-β polypeptides, separately isolated from *R. spheroides* forms a subunit-type complex with Zn-BChl *a* absorbing at 812 nm in 0.78% OG at 25 °C and forms a LH1-type complex with Zn-BChl *a* absorbing at 859 nm when cooling to 4 °C (Table 1), consistent with BChl *a*.[10] However, the native LH-β alone forms the subunit-type complex with Zn-BChl *a* absorbing at



Scheme 2. Amino acid sequences of the native LH-β polypeptide and synthetic hydrophobic polypeptides.

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

Polypeptides	Qy band / nm		CD maxima / nm (10^{-4} θ)	
	25 °C	4 °C	4 °C	
type 1	810	834	838 (33)	817 (-36)
type 2	809	834	837 (34)	819 (-27)
type 3	810	810	816 (7.8)	798 (-21)
type 4	807	807	816 (11)	798 (-36)
LH-β[b]	814	817	822 (-6.2)	
LH-α and LH-β[b]	812	859	867 (7.7)	832 (-5.6)

[a] [Zn-BChl *a*] = 2.42×10^{-6} mol dm^{-3} and [polypeptides] = 3.45×10^{-6} mol dm^{-3} in 0.78% OG solution (phosphate buffer ; pH 7.5). [b] LH polypeptides separately isolated from *R. sphaeroides*.

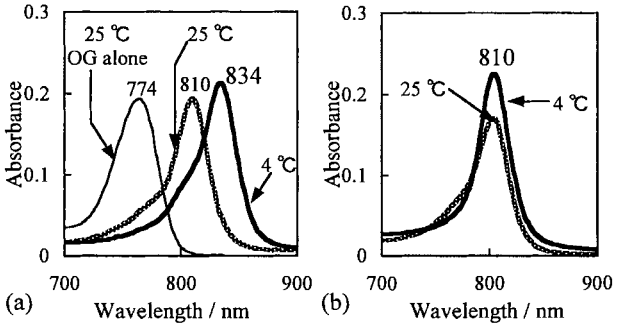


Figure 1. UV-vis. spectra of Zn-BChl *a* in the presence of type 1 (a) or type 3 (b) in 0.78% OG solution.

817 nm at 4 °C (Table 1) as well as with BChl *a*.[10] Figure 1(a) shows UV-vis. absorption spectra of the Qy band for Zn-BChl *a* in the presence and absence of type 1 in OG micelle. The Qy band of Zn-BChl *a* in the absence of type 1 was observed at 774 nm, analogous to the Qy band of Zn-BChl *a* monomer in acetone. Interestingly, in the presence of type 1 the Qy band was red-shifted to 810 nm in 0.78% OG at 25 °C and further red-shifted to 834 nm when cooling the sample to 4 °C, implying the formation of the subunit-type complex at 25 °C and the formation of the LH1-type complex at 4 °C (Table 1). Furthermore, a large split CD signal of Zn-BChl *a* due to the presence of type 1 was observed around the Qy absorption band at 4 °C, consistent with that in the LH1-type complex (Table 1).[1] These UV-vis. and CD spectral data for Zn-BChl *a* was also observed in the presence of type 2 which was substituted from W(-10) to F (-10) in the hydrophobic core of the type 1(Table 1). These results indicate that type 1 organizes Zn-BChl *a* complex analogous to the LH1-type complex where the W(-10) residue of type 1 as well as the N-terminal segment of the native LH-β are not crucial in forming the LH1-type complex. This is the first report that Zn-BChl *a* complex can be organized by the synthetic hydrophobic 1α-helix polypeptide such as type 1 in OG micelle, where Zn-BChl *a* is very stable in comparison to BChl *a*.

Alternatively, to further examine the effect of an amino acid residue in type 1 on forming the LH1-type complex, molecular assemblies of Zn-BChl *a* by types 3 and 4 were examined. Figure 1(b) shows UV-vis. absorption spectra of the Qy band for Zn-BChl *a* in the presence and absence of type 3 which is substituted from W(+9) to F(+9) in the C-terminal segment of the type 1. The Qy band of Zn-BChl *a* was red-shifted to 810 nm due to the presence of type 3 in 0.78% OG at 25 °C, consistent with the formation of subunit-type complex in the presence of the type 1 (Table 1). However, no further red-shift of the Qy band was observed when cooling the sample to 4 °C, indicating no formation of the LH1-type complex.[10] Interestingly, a large split CD signal of Zn-BChl *a* due to the presence of type 3 was observed around the Qy absorption band at 4 °C, implying that a stable dimer complex such as the subunit-type complex was formed due to their hydrophobic interactions (Table 1). Similar UV-vis. and CD spectral data for Zn-BChl *a* was also observed due to the presence of type 4 which was substituted from W(+6) to F(+6) in the C-terminal segment of the type 1 (Table 1). Comparison of these UV-vis. and CD spectral data for Zn-BChl *a* between these synthetic polypeptides reveals that two of the amino acid residues, W(+9) and W(+6) in the C-terminal segment of type 1 are essential to form the LH1-type complex.[10] These results imply that C3 acetyl carbonyl group of Zn-BChl *a* may binds with W(+9) and W(+6) in the C-terminal of LH polypeptides through hydrogen-bondings to form LH1 complex.[2]

In conclusion, synthetic hydrophobic 1α-helix polypeptides assemble Zn-BChl *a* complex in OG micelle analogous to the LH1-type complex from phtosynthetic bacteria, depending upon the temperature and polar amino acid sequences in the C-terminal segment. Appropriate analogues of these synthetic polypeptides are useful in constructing an artificial LH complex with Zn-BChl*a* as well as in providing an insight into the effect of polypeptide structure on the LH complex.

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