Molecular Assembly of Bachteriochlorophyll *a* Using Light-harvesting Model 1α-Helix Polypeptides and 2α-Helix Polypeptide with Disulfide-linkage

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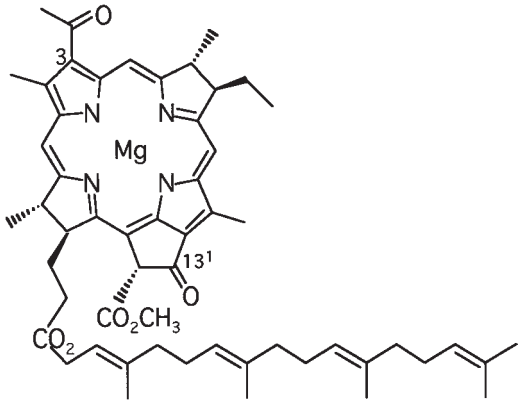
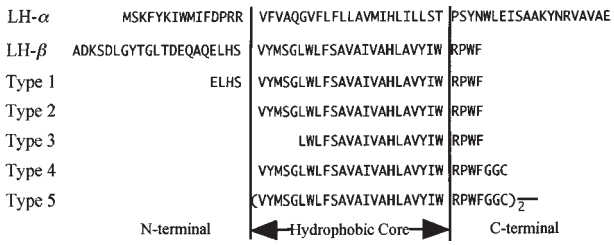
UV-vis. absorption and CD spectral data indicate that the minimum amino acid sequence in the N-terminal segment of the light-harvesting (LH) polypeptides of photosynthetic bacteria necessary for the LH1-type complex-formation was that of the type 2, of our synthetic hydrophobic polypeptides (Scheme 2). Stable BChl *a* complex in the presence of a 2α-helix polypeptide with disulfide linkage, was obtained in lipid bilayers as well as in n-octyl-β-D-glucopyranoside (OG) micelles, analogous to the subunit complex, B820, of the LH I complex of photosynthetic bacteria.

It is interesting to note that light-harvesting (LH) polypeptides organize a bacteriochlorophyll *a* (BChl *a*) complex so that an efficient energy-transfer may occur in photosynthetic bacteria.[1] It is considered that the Mg atom in the BChl *a* coordinates with the histidine residue in the hydrophobic core of the LH polypeptides, and tryptophan or other polar amino acid residues in the C-terminal of the LH polypeptide may bind with the C3 acetyl and C13[1] keto carbonyl of BChl *a* through hydrogen-bonds, to form the LH complex.[1,2] Works from several laboratories has demonstrated assemblies of porphyrins with synthetic polypeptides to produce artificial hemoproteins or LH complex models.[3] However, there has been no study of the molecular assembly using 2α-helix synthetic polypeptides with a disulfide-linkage to try to organize an artificial LH complex in lipid bilayers.

In this paper, we examine the molecular assembly of BChl *a* (Scheme 1) with synthetic hydrophobic polypeptides (Scheme 2) in lipid bilayers as well as in n-octyl-β-D-glucopyranoside (OG) micelles. We selected these α-helical polypeptides. Types 1–5, which have similar amino acid sequences to the hydrophobic core in the native LH-β polypeptide from the photosynthetic bacterium, Rb. sphaeroides, because the LH-β polypeptide forms a stable BChl *a* complex analogous to the LH I subunit-type complex.[2,4] Types 1–3 were synthesized to see the effect of the smallest amino acid sequence in the N-terminal segment required to form the LH complex, while Types 4–5 were synthesized to investigate the effect of the disulfide-linkage in the C-terminal segment on the formation of the LH complex.[5] The native LH-α and -β polypeptides were separately isolated from the LH I complex of Rb. sphaeroides and BChl *a* was obtained as described previously.[4] The molecular assembly of BChl *a* using the synthetic polypeptides was carried out according to the reconstitution method reported previously.[4]



Scheme 1. Structure of BChl *a*.



Scheme 2. Amino acid sequences of synthetic 1α-helix and 2α-helix polypeptides.

Table 1 shows the Qy absorption band and the CD signal of BChl *a* in the presence of the synthetic hydrophobic polypeptides. It is clear from Table 1 that the Qy band of the BChl *a*-monomer is observed at 777 nm in acetone while the Qy band in the presence of Type 1 or 2 is red-shifted to 818 nm in 0.78% OG at 25 °C. This is consistent with the subunit-type complex formation as formed previously with the LH-α/-β polypeptides as well as using the LH-β alone at 25 °C.[6] On cooling the sample to 4 °C, the Qy band

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

Polypeptides	The Qy absorption band/nm		CD spectra 4 °C	
	25 °C	4 °C	θ (10^{-4} deg cm^{-2} dmol^{-1})	
Type 1	818	848	849 (41)	828 (−34)
Type 2	818	843	841 (51)	822 (−30)
Type 3	777[d] 850	777[d] 850	—[e]	
Type 4	818	843	854 (6.0)	813 (−7.0)
Type 5	814	814	823 (10)	802 (−20)
LH-β	821	823	828 (−4.5)	
LH-α and LH-β	821	873	888 (8.2)	855 (−6.1)
none[b]	777[d] 850	777[d] 850	—[e]	
none[c]	777	777	no signal	

[a]Measured in 0.78% OG solution (phosphate buffer pH 7.5), [Bacteriochlorophyll *a*] = 2.4×10^{-6} mol dm^{-3}, [polypeptides] = 3.4×10^{-6} mol dm^{-3}. [b]In 0.78% OG solution. [c]In acetone. [d]Broad peaks were observed. [e]No clear signal was observed.

is further red-shifted to 848 or 843 nm for Type 1 or 2, respectively, consistent with the LH I-type complex formation using the native LH-α/-β polypeptides.[2,4] However, no clear red-shift of the Qy band due to the presence of Type 3 is observed at 25 and 4 °C. Rather broad absorptions around 777 and 850 nm are seen, analogous to these of the aggregated BChl *a* complex in the OG micelle (Table 1). This result is attributed to the fact that the α-helicity content of Type 3 is too low to form the complex. Furthermore, a large split CD signal of BChl *a* in the presence of Type 2 or Type 1 is observed around the Qy band at 4 °C. This is again with the signal seen in the LH I-type complex using the LH-α/-β polypeptides (Table 1). This UV-vis. absorption and CD spectral data indicate that the minimum amino acid sequence in the N-terminal segment of the LH polypeptides necessary for the formation of the LH I-type complex is that of the Type 2, among our synthetic hydrophobic polypeptides.[6]

To see the effect of the amino acid sequence in the C-terminal segment of the LH polypeptides on the formation of the LH complex, we examined the molecular assembly of BChl *a* with Type 4 or 5. Figure 1 shows the Qy absorption band of BChl *a* in the presence of Type 4 or 5 in 0.78% OG micelle at 4 °C. The Qy band is red-shifted to 843 nm in the presence of Type 4, similar with that seen in the presence of Type 1 or 2 (Table 1). Interestingly, a similar red-shift of the Qy band is not observed in the presence of Type 5, with the result that the Qy band becomes blue-shifted and sharpened dramatically at 814 nm, especially in comparison to that seen in the presence of Type 4 in 0.90% OG at 25 or 4 °C. The Qy band at 814 nm is analogous to that in the subunit-type complex, where the subunit-type complex is usually disorganized by the high concentration of OG.[4] This result reveals that the disulfide-linkage in the C-terminal segment obstructs formation of a LH I-type complex. Interestingly, the CD signal-intensity of the Type 5 peptide at 208 nm decreases due to the presence of BChl *a* in the OG micelle at 4 °C, while the intensity at 222 nm increases. This implies that cooperative packing interactions between the α-helical polypeptides occur due to the presence of BChl *a*.[7] Furthermore, a large split-CD signal in the Qy band of BChl *a* in the presence of Type 5, in comparison to those in the presence of Type 4, also supports the idea of cooperative packing interactions between Type 5 and BChl *a* (Table 1). These UV-vis. and CD data reveal that the disulfide-linkage at the C-terminal segment of Type 5 strongly influences the stabilization of the subunit-type complex formation.[2,4]

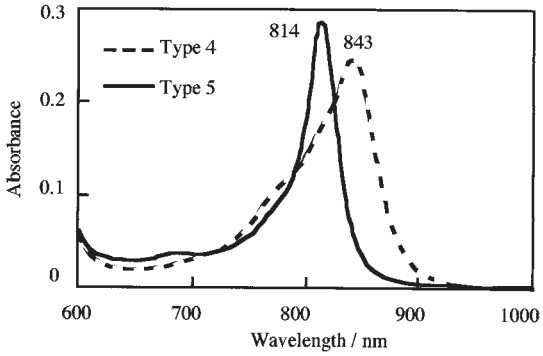


Figure 1. UV-vis. spectra of BChl *a* in the presence of Type 4 or Type 5 in 0.78% n-octyl-β-D-glucopyranoside (OG) solution at 4 °C. Concentrations were as follows: [BChl *a*] = 2.4×10^{-6} mol dm^{-3}, [polypeptides] = 3.4×10^{-6} mol dm^{-3}.

To examine further the effect of the disulfide-linkage in the C-terminal segment of Type 5 on the LH-type complex formation, the molecular assembly of BChl *a* with Type 4 or 5 was carried out in lipid bilayers as follows.[8] 0.78% OG solution containing the complex of Type 5 or 4 with BChl *a* was mixed with a solution of liposomal membranes containing egg yolk-phosphatidylethanol-amine, dipalmitoylphosphatidylglycerol, phosphatidylglycerol, cholesterol (2 : 1 : 1 : 4) and calcein. Then, the mixed solution was gel-filtered by a Sephadex G-50 column to remove the complex from the external vesicle surface. Proof of insertion of the complex into the lipid bilayers was indicated by the change in the Qy band of BChl *a*, and where little or no leakage of calcein embedded in the inner liposomal membrane was observed. For example, after the gel filtration, the Qy band was red-shifted from 777 to 814 nm due to the presence of Type 5 in the lipid bilayers, analogous to the subunit-type complex in the OG micelle. However, the Qy band showing the complex formation was not observed in the presence of Type 4, where the complex was destroyed during the gel filtration. This is the first report that a stable BChl *a* complex due to the presence of Type 5 can be obtained in lipid bilayers as well as in the OG micelles as shown in the graphical abstract.

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5 These polypeptides were synthesized by the Fmoc method using a peptide synthesizer as described previously. These polypeptides were analyzed by TOF-MS to give the expected molecular mass. CD spectra of these polypeptides showed strong *a*-helicity for each polypeptide except Type 3 (*a*-helix content, Type 1: 29%, Type 2: 25%, Type 3: 11%, Type 4: 30%, Type 5: 50%, the native LH-β: 50%).
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